



## 25 **Abstract**

26

27 The global occurrence of persistent organic pollutants (POPs) continuously contributes to  
28 their accumulation also in remote areas such as the Antarctic Ocean. Antarctic fish, which  
29 hold high trophic positions but appear to possess low endogenous elimination rates for  
30 chemicals, are expected to bioaccumulate POPs with rising anthropogenic pollution.  
31 Using a chemical-analytical method, we measured concentrations of PCBs, PBDEs, HCBs,  
32 HCH and DDTs and determined toxic equivalents (TEQs) and bioanalytical equivalents  
33 (BEQs) in muscle and ovaries of Antarctic icefish caught in the Southern Ocean around  
34 Elephant Island. We used two species with different feeding habits and trophic web positions:  
35 the planktivorous *Champsocephalus gunnari* and the piscivorous *Chaenocephalus aceratus*.  
36 Our results revealed higher contaminant levels in ovary than in muscle tissues of both  
37 species. Most analytes concentrations and the TEQs (0.2-0.5) and BEQs (0.2) were lower as  
38 in temperate species. Comparison with literature data points to higher PCB (20-22 ng g<sup>-1</sup> lipid  
39 weight (lw)) and DDT (7-19.5 ng g<sup>-1</sup> lw) concentrations than those measured in icefish in the  
40 90's. For the other contaminants, we could not identify temporal trends. We found a higher  
41 bioaccumulation of contaminants, particularly HCB and DDTs, in *C. aceratus* (6.2 & 19.5 ng  
42 g<sup>-1</sup> lw, respectively) than in *C. gunnari* (3.8 & 7.0 ng g<sup>-1</sup> lw, respectively). However, there  
43 was no general species-specific accumulation pattern of the different toxicant classes between  
44 the two icefish. Thus, the expected link between contaminant burdens of *C. aceratus* and *C.*  
45 *gunnari* and their ecological traits was only weakly supported for these species.

46

## 47 **Highlights**

48

- 49 - PCB and DDT concentrations in icefish are higher than those measured in the late 90s
- 50 - Mature, female icefish possess higher contaminant levels in ovaries than in muscle
- 51 - POP levels are similar in fish from different sampling sites around the Antarctic  
52 Peninsula
- 53 - Piscivorous icefish display a higher toxicant accumulation than planktivorous icefish
- 54 - Bioanalytical results (BEQs) were similar to chemical analytical calculations (TEQs)  
55 in icefish

56

## 57 **Keywords**

58 Icefish, bioaccumulation, persistent organic pollutants, polychlorinated biphenyls (PCBs),  
59 toxic equivalents (TEQs), DR CALUX bioanalytical equivalents (BEQs)

60  
61

## 62 **1. Introduction**

63

64 Antarctica has been less affected by human influences than other continents for a long time,  
65 however, contamination with anthropogenic contaminants, in particularly persistent organic  
66 pollutants (POPs) has increased progressively (Nash, 2011; UNEP/AMAP, 2011). Nowadays,  
67 Antarctica serves as a major sink for highly persistent contaminants. Long-range atmospheric  
68 transport, together with global distillation processes and cold condensation, are considered to  
69 be the main mechanisms for the progressive contamination of the Antarctic ecosystem,  
70 together with local sources such as fishing, tourism and research activities (Simonich and  
71 Hites; Feely et al., 2008; Nash, 2011). Particularly POPs such as polychlorinated biphenyls  
72 (PCBs) and polybrominated diphenyl ethers (PBDEs), and amongst them also the formerly  
73 used insecticides such as  $\gamma$ -hexachlorocyclohexane ( $\gamma$ -HCH) and *p,p'*-DDT are ubiquitous  
74 pollutants with a wide application spectrum. For example, polymer additives such as flame  
75 retardants are found globally in building material, furniture, paint, textiles or plastics, which  
76 are also used in Antarctic bases and vessels cruising in these regions (Hale et al., 2008;  
77 Kohler et al., 2008). This worldwide abundance and the high persistence of POPs  
78 continuously contribute to an accumulation in the ice masses and biota of polar regions  
79 (Wania and Mackay, 1993; Chiuchiolo et al., 2004; Goerke et al., 2004; Corsolini et al.,  
80 2007; Bargagli, 2008; Borghesi et al., 2008; Borghesi et al., 2009; Xie et al., 2011; Wolschke  
81 et al., 2015).

82

83 Furthermore, those lipophilic organic chemicals have a high potential to bioaccumulate in  
84 aquatic biota, and particularly in Antarctic species, which generally possess low endogenous  
85 elimination rates for those chemicals (Strobel et al., 2015). Additionally, it is expected that  
86 climate warming will lead to the release of those pollutants trapped in glaciers and sea-ice,  
87 which additionally contributes to increasing POP levels in the tissues of Antarctic animals  
88 (Weber and Goerke, 2003; Bogdal et al., 2010; Schmid et al., 2010; van den Brink et al.,  
89 2011; Cabrerizo et al., 2013; Goutte et al., 2013). As global chemical usage continues to  
90 grow, including the usage of persistent and bioaccumulative compounds, it is to be expected  
91 that contaminant intake into Antarctica will further increase in the future.

92

93 The endemic, Antarctic notothenioid fish are evolutionary well-adapted to the cold and stable  
94 environment of the Southern Ocean. Adaptations involve e.g. high amounts of tissue lipids,  
95 slow growth rates, long life spans and slow metabolism and elimination rates for xenobiotics  
96 (Mintenbeck et al., 2012; Strobel et al., 2015). All these factors may favor the  
97 bioaccumulation of lipophilic contaminants in the tissues of those fish.

98

99 Lipophilic, non-charged contaminants like PCBs can be taken up via a physico-chemically  
100 driven, passive partitioning of the chemicals from the water phase into the lipid phase of the  
101 organism and thereby accumulate differentially in tissues or fish species, depending on their  
102 lipophilicity of the chemical and the lipid content or composition of the tissues (Nichols et  
103 al., 2013). In fact, tissue-specific differences of POP levels are reported for notothenioid  
104 species (Lana et al., 2014). Furthermore, bioaccumulation and biomagnification can be  
105 related to an organism's habitat and its trophic position within the food web (Corsolini et al.,  
106 2002a; Weber and Goerke, 2003; Chiuchiolo et al., 2004). Particularly for highly lipophilic  
107 compounds, oral uptake via the prey contributes by a major extent to the bioaccumulation of  
108 toxicants in the tissues of a species. For example, Weber and Goerke (2003) found higher  
109 contaminant levels in the piscivorous icefish *Chaenocephalus aceratus* than in the  
110 planktivorous icefish *Champscephalus gunnari*, which was apparently related to the  
111 different food spectra of the two notothenioids (Goerke et al., 2004). Such studies highlight  
112 that POPs are transferred within the Antarctic food web, leading to increasing POP  
113 concentrations along the food chain up to high concentrations in top level predators  
114 (Wolschke et al., 2015).

115

116 Considering the trends of rising POP concentrations in Antarctica (UNEP/AMAP, 2011 and  
117 their high potential to exert toxic effects in marine biota (Nash, 2011), it is very important to  
118 keep monitoring body burdens of the fish living in the Southern Ocean. Yet, we are far from  
119 having a solid understanding of the contamination status, time trends, the diversity of  
120 contaminants or their toxicity potential in Antarctic biota (UNEP, 2002; UNEP/AMAP,  
121 2011). Particularly the bioaccumulation pattern of contaminants such as the polybrominated  
122 flame retardants (PBDEs) or insecticides (e.g. DDT) have hardly been measured in icefish.

123

124 The aim of this study was to determine levels of selected POPs in muscle and ovary tissue of  
125 two white-blooded Antarctic notothenioid species in the study area around Elephant Island,

126 the South Shetland Islands and the Antarctic Peninsula, and to estimate temporal trends of  
127 fish POP levels in this area. The analytically measured contaminant concentrations were  
128 converted into toxic equivalents (TEQs) using standardized values. In order to examine  
129 whether food web position or tissue lipid content has an influence on chemical  
130 concentrations, we analyzed lipid content and POP levels in fish species of the same family  
131 but with different feeding habits and trophic web positions, namely the planktivorous *C.*  
132 *gunnari* and the piscivorous *C. aceratus*.  
133 Extending the classical contaminants, this study includes polybrominated flame retardants  
134 (PBDEs) and insecticides (i.e. DDT, HCB). In addition to chemical analytics, we also applied  
135 bioanalytics using the DR-CALUX assay (Murk et al., 1996; Kuiper et al., 2006) to assess the  
136 total accumulated dioxin-like activity in the fish tissues.

137

138

## 139 **2. Methods**

140

### 141 2.1 Study species

142 Two species of the *Channichthyidae* (white-blooded icefish) were fished with bottom trawls  
143 down to 500 m, during a cruise with the research vessel ‘Polarstern’ (ANTXXVIII/4, March  
144 13 to April 9, 2012; [http://expedition.awi.de/expedition/ANT-](http://expedition.awi.de/expedition/ANT-XXVIII/4?alias=PS79#mapChart)  
145 [XXVIII/4?alias=PS79#mapChart](http://expedition.awi.de/expedition/ANT-XXVIII/4?alias=PS79#mapChart)) at different, closely located sampling sites around Elephant  
146 Island and the South Shetland Islands (61.1°S, 55.1°W). Only fish netted alive and without  
147 macroscopically visible damage were used for tissue sampling. The planktivorous mackerel  
148 icefish, *C. gunnari*, shows a mainly benthopelagic feeding mode, while the piscivorous  
149 Scotia Sea icefish *C. aceratus* is predominantly a benthos feeder (Weber and Goerke, 2003).  
150 Persistent organic pollutants were analyzed in muscle and ovary tissue of mature (stage III-  
151 IV), female fish only. Sex and maturity stage of the fish were verified histologically. Animal  
152 weight and length are given in Table 1. All tissue samples were wrapped in aluminum foil  
153 and stored immediately at -20 °C until used for analysis.

154

### 155 2.2 Sample preparation

156

157 Muscle and gonad samples were defrosted, cut into pieces and lyophilized at 33 Pa for 72  
158 hours until constant weight. Dried tissue of muscle (5-10 g) or gonads (0.5-2 g) was ground

159 with anhydrous sodium sulfate and quartz sand in a ceramic mortar and pestle to obtain a fine  
160 powder.

161 This homogenate was then Soxhlet-extracted with a speed-extractor (E-914, Büchi,  
162 Switzerland) in 120 mL extraction cells at a constant temperature and pressure of 100 °C and  
163 100 bar, respectively (4 cycles, hold time 10 min., discharge 4 min), with ~150 ml *n*-  
164 hexane/dichloromethane 1:1 (v:v) (Hartmann, 2013). The extract was concentrated in a  
165 Syncore evaporator (Büchi, Switzerland) and let dry completely by applying a gentle  
166 nitrogen stream. The residue accounted for the fat content of the sample. <sup>13</sup>C<sub>12</sub> labeled  
167 internal standards (Schmid et al., 2007) were added to the samples and after the addition of 2  
168 – 3 mL of *n*-hexane the solution was treated with 3 ml oleum (7% SO<sub>3</sub> in conc. sulfuric acid).  
169 After centrifugation for 3 min at 5'000 rpm, the solvent layer with the lipophilic target  
170 analytes was removed and the remaining suspension was re-extracted two times more with *n*-  
171 hexane. The pooled extracts were concentrated to 0.5 mL in a rotary evaporator at 45 °C and  
172 300 mbar. Subsequently, the extract was purified on a multilayer mini silica gel column (from  
173 top to bottom: 0.25 g anhydrous sodium sulfate, 0.25 g silica gel 60 with 44% sulfuric acid  
174 and 0.25 g silica gel 60 activated at 130 °C). The sample was applied on the column and  
175 eluted with 5 ml *n*-hexane, followed by 5 mL *n*-hexane/dichloromethane 1:1 (v:v). The eluate  
176 was concentrated using a rotary evaporator to 0.5 mL. After transfer to a mini GC-Vial the  
177 volume was further reduced to 30 µL by the application of a gentle stream of nitrogen at  
178 room temperature. Finally the recovery standard <sup>13</sup>C<sub>12</sub> labeled PCB 70 was added. Samples  
179 were stored in toluene at -20 °C until analysis. Method blank levels for the whole analytical  
180 procedure were determined in duplicates (Table 1 and A.2).

181

### 182 2.3 Chemical analysis

183

184 PCBs (indicator PCB 28, 52, 101, 138, 153, and 180; dioxin-like PCBs 77, 81, 105, 114, 118,  
185 123, 126 156, 157, 167, 169, and 189), DDT (*o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE),  
186 hexachlorobenzene (HCB),  $\gamma$ -hexachlorocyclohexane ( $\gamma$ -HCH), and PBDEs (BDE 28, 47, 99,  
187 100, 153, 154, 183, and 209) were included in this study. Quantitative determination of the  
188 target analytes in the extracts was achieved by gas chromatography/high resolution mass  
189 spectrometry (GC/HRMS). Analyses were carried out on a Finnigan MAT95 high-resolution  
190 mass spectrometer (Thermo Finnigan MAT, Bremen Germany) coupled to a Finnigan Trace

191 GC Ultra equipped with a Triplus auto sampler (Thermo Electron Corporation, Waltham,  
192 MA, USA). Samples were injected in splitless mode (splitless time 30 s) at an injector  
193 temperature of 260 °C. For the gas chromatographic separation a RTX5 Sil-MS column (30  
194 m × 0.25 mm, film thickness 0.10 µm) was used with helium as carrier gas at a pressure of  
195 100 kPa. The following temperature programs were used for the different compound classes.  
196 For the PCBs, the initial column temperature was 100 °C. After 0.5 min, the temperature was  
197 ramped at 20 °C/ min to 180 °C, followed by 3 °C/ min to 250 °C, and 20 °C/ min to 300 °C.  
198 For pesticides, the initial column temperature was 100 °C. After 0.5 min, the temperature was  
199 ramped at 10 °C/ min to 160 °C, followed by 4 °C/ min to 240 °C, and 20 °C/ min to 300 °C.  
200 For the PBDEs, the initial column temperature was 100 °C. After 0.5 min, the temperature  
201 was ramped at 20 °C/ min to 220 °C, followed by 6 °C/ min to 300 °C, and 10 °C/ min to 320  
202 °C. The ion source was operated at 220 °C, the electron energy was 70eV, and the mass  
203 spectrometer was tuned to a mass resolution of 8000-10000. The two most abundant signals  
204 of the molecular ion cluster of the analytes and the <sup>13</sup>C<sub>12</sub> labeled internal standards were  
205 recorded in the single ion monitoring mode.

206

207 The analytes were identified by comparing the retention times with those of the labeled  
208 internal standards. Quantification was based on peak areas of the analytes and the labeled  
209 reference compounds with known concentrations. More details about the method are  
210 available in the literature (Zennegg et al., 2003; Schmid et al., 2007).

211 For the calculation of TEQs, the dioxin-like PCBs were determined in the same way, but as  
212 part of the sample extract was used later on for the analysis by the bioassay (DR-CALUX),  
213 no <sup>13</sup>C<sub>12</sub>-labeled internal dl-PCBs standards could be used for the quantification, as the  
214 isotope labeled analogues exhibit similar activity in the DR-CALUX. Therefore, quantitative  
215 determination of dl-PCBs was based on the <sup>13</sup>C<sub>12</sub> labeled indicator PCBs used as internal  
216 standards and previously determined response factors to native dl-PCBs.

217 The blank values (in ng g<sup>-1</sup> lipid weight) were all below the compound concentrations and are  
218 given in Table 1 and A.1.

219 The limit of detection (LOD) and the limit of quantification (LOQ) were set by definition at  
220 signal to noise ratios of greater than three (s/n ≥ 3) and ten (s/n ≥ 10) respectively. All glass  
221 ware used were cleaned with strongly alkaline detergents and backed out overnight in a  
222 ceramic oven at 450 °C. Directly before use, the glass ware was rinsed with solvents (*n*-  
223 hexane, dichloromethane).

224

## 225 2.4 Determination of dioxin-like toxic equivalents (TEQs)

226

227 In order to assess total dioxin-like activity in the fish tissues, we calculated the 2,3,7,8-  
228 tetrachlorodibenzo-*p*-dioxin (TCDD) equivalent (TEQ) concentrations of dl-PCBs in the  
229 muscle and gonad extracts of both icefish species using Toxic Equivalency Factors (TEFs)  
230 proposed by the World Health Organization (WHO) for fish (Van den Berg et al., 1998).  
231 TEQs were calculated as the sum of the TEFs of all dl-PCBs listed in Table A.1. Although  
232 the TEF values are not derived from studies with Antarctic fish, since TEFs are only available  
233 for salmonids (Van den Berg et al., 1998) they provide a useful tool for a reasonable estimate  
234 of toxicity effects of PCBs on Antarctic fish.

235

## 236 2.5 Determination of bioequivalent values (BEQs)

237

238 The same extracts that were used for chemical analysis were also used in a standard bioassay,  
239 the DR-CALUX (Dioxin Responsive Chemically Activated Luciferase Gene Expression)  
240 assay. This cell and receptor based reporter gene bioassay measures the binding of dioxin-like  
241 HAHs, e.g. dioxin-like PCBs (dl-PCBs), to the Aryl hydrocarbon receptor (AhR) via  
242 activation of a reporter gene. The assay was performed by BioDetection Systems b.v.  
243 (Amsterdam, The Netherlands).

244

245 Bioassay-derived TEQ values, or bioequivalent values (BEQs), were compared with TEQ  
246 values calculated from the chemical analytical data. This comparison revealed if there was a  
247 significant higher activity than predicted from the chemical analysis. Such a combined  
248 approach has been repeatedly used in studies on chemical contamination of the Arctic region  
249 (Letcher et al., 2010), but it has not been used in studies with Antarctic fish so far.

250

## 251 2.5 Calculations and statistics

252

253 All analyses include the lipid content of the respective tissue. POP concentrations were  
254 normalized against both lipid weight (lw, ng g<sup>-1</sup>) and fresh weight (fw, ng g<sup>-1</sup>) of the tissue  
255 and provided as mean values ± standard error of the mean (sem). Toxicant concentrations  
256 were compared among icefish species and tissues using ANOVA with Tukey post-hoc test.



257 Influence of sampling site, fish weight and length, maturity stage and tissue lipid content was  
258 tested with ANOVA. Data were considered to be statistically significant at  $p < 0.05$ . Normal  
259 distribution of data was tested with Kolmogorov-Smirnov and equality of variances with  
260 Bartlett's test. All statistical tests were performed with STATISTICA 12, StatSoft, Inc., and  
261 GraphPad Prism 5, GraphPad Software, Inc.

262

263

### 264 3. Results

265

#### 266 3.1 Interspecies comparison of contaminant patterns and levels

267

268 In this study, we measured lipid content, PCB, DDT, HCB,  $\gamma$ -HCH, and PBDE concentrations  
269 in both muscle and ovary tissue of female, mature *C. gunnari* and *C. aceratus* from spatially  
270 closely located sampling sites around Elephant Island and the South Shetland Islands. We  
271 express the compound concentrations on lipid weight and the fresh weight basis.

272 Sampling site, maturity stage and tissue lipid content of the fish showed no correlation to the  
273 accumulation of any of the compounds analysed in this study.

274 All samples contained detectable levels of the target compounds, which are presented in  
275 Table 1. Analytes concentrations were about 15 - 110 times higher when calculated per lipid  
276 weight than based on tissue fresh weight.

277 Overall, the PCBs were the predominant group among all compounds analysed in our study  
278 (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  $20.0 \pm 4.3$ , *C. gunnari* ovaries  $47.8 \pm$   
279  $10.2$ , *C. aceratus* muscle  $21.9 \pm 4.9$ , *C. aceratus* ovaries  $31.9 \pm 7.3$ ), followed by the DDTs  
280 (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  $7.0 \pm 1.0$ , *C. gunnari* ovaries  $6.9 \pm 1.1$ , *C.*  
281 *aceratus* muscle  $19.4 \pm 8.1$ , *C. aceratus* ovaries  $17.8 \pm 3.9$ ), HCB (mean values  $\pm$  sem ( $\text{ng g}^{-1}$   
282 lw): *C. gunnari* muscle  $3.8 \pm 0.5$ , *C. gunnari* ovaries  $2.8 \pm 0.4$ , *C. aceratus* muscle  $6.2 \pm 0.9$ ,  
283 *C. aceratus* ovaries  $7.3 \pm 2.0$ ), the PBDEs (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  
284  $4.1 \pm 0.5$ , *C. gunnari* ovaries  $0.7 \pm 0.1$ , *C. aceratus* muscle  $5.1 \pm 0.8$ , *C. aceratus* ovaries  $10.0$   
285  $\pm 1.9$ ) and  $\gamma$ -HCH (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  $0.6 \pm 0.2$ , *C. gunnari*  
286 ovaries  $1.3 \pm 0.3$ , *C. aceratus* muscle  $0.6 \pm 0.2$ , *C. aceratus* ovaries  $1.2 \pm 0.5$ ) (Figure 1).

287 Within the PCBs, only PCB 28 and 101 were significantly different between the ovaries of *C.*  
288 *gunnari* and *C. aceratus* (Table 1). When we calculated the percentage contribution of the  
289 individual PCB congeners to  $\sum$ PCBs (lw) of both muscle and ovary tissue, there was no

290 difference in PCB congener composition between the two icefish species (two-way  
291 ANOVA).  
292 Among the PCBs, PCB 153 contributed by 28% in muscle and by 26% in ovaries to all PCBs  
293 in both species. The second-most abundant PCBs were PCB 138 (~26%) and 101 (~21%)  
294 (Figure 2).

295  
296 In the ovaries, levels of *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT (per lw) were significantly  
297 higher in *C. aceratus* compared to those of *C. gunnari*. Also *p,p'*-DDT concentrations in the  
298 muscle of *C. aceratus* were higher than those in muscle of *C. gunnari* (per lw).  $\Sigma$ DDT (per  
299 lw) was significantly higher in muscle and ovaries of *C. aceratus* than in *C. gunnari* (Figure  
300 1). Among the DDTs, *p,p'*-DDE showed the highest concentration of up to 57% in muscle of  
301 *C. aceratus*. *p,p'*-DDE tended to be higher in muscle tissue, while *p,p'*-DDT was slightly  
302 higher in ovary tissue in both species. *o,p'*-DDT was slightly higher in *C. gunnari* with  
303 ~22%, while it was only ~16% in *C. aceratus*. Detailed information on the contribution of all  
304 individual compounds measured in this study is given in Table A.2 in the appendix.

305  $\gamma$ -HCH was only different between the muscle tissues of the two icefish (on fw basis).  
306 HCB concentrations per lipid weight in both muscle and ovaries were higher in *C. aceratus*  
307 than in *C. gunnari* (Figure 1). Except HCB, none of the analysed compounds exhibited a  
308 correlation with fish weight or length. HCB showed a significant linear dependence of fish  
309 weight and length in *C. aceratus*, except in the ovaries ( $R^2=0.723$ ).

310  
311 In both species, the PBDEs were dominated by BDE 47 (~59% in muscle, ~54% in ovaries),  
312 followed by ~20% BDE 99. On both the lipid and the fresh weight basis, BDE 183 and 197  
313 were significantly different between the two tissues types and fish species. Also BDE 99  
314 showed different concentrations between the ovaries of *C. aceratus* and *C. gunnari* on the  
315 lipid basis (Table 1, Table A.2, Figure 2).

316  
317 3.2 Comparison of contaminant patterns and levels in different tissue

318  
319 Lipid content was significantly higher in the ovaries of *C. gunnari* than in the ovaries of *C.*  
320 *aceratus*. *C. gunnari* exhibited significantly higher lipid content in ovaries than in their  
321 muscle tissue, while this was not the case for *C. aceratus* (Table 1).

322 A comparison between the compound concentrations in muscle and ovary tissue for each  
323 icefish species revealed higher HCB concentrations in ovary than in muscle tissue of *C.*

324 *gunnari*. Concentrations of  $\gamma$ -HCH and DDTs in muscle and ovary tissue (per fresh weight)  
325 were significantly different in both species (Table 1).

326 In *C. aceratus*, all PCB concentrations and most of the PBDEs were different from each other  
327 in both muscle and ovary tissue. In case of dl-PCB congeners, the  $\sum$ dl-PCBs were higher in  
328 muscle of *C. gunnari* than in muscle *C. aceratus* on both lipid and fresh weight basis (Table  
329 A.1).

330 In *C. gunnari*, all PBDEs apart from congener 183 did differ between muscle and ovary  
331 tissue. In contrast, only PCB 28 and 180 were different between the tissues of *C. gunnari*, all  
332 other PCB congeners showed no significant tissue differences (Table 1, Figure 1).

333

### 334 3.3 Toxic equivalents (TEQs)

335

336 The toxic equivalent levels of dioxin-like PCBs ( $WHO_{PCB}\text{-TEQ g}^{-1}$  fw) were higher in the  
337 muscle of *C. gunnari* ( $0.5 \pm 0.1$ ) than in *C. aceratus* ( $0.2 \pm 0.1$ ), while *C. aceratus* exhibited  
338 higher TEQs ( $0.5 \pm 0.9$ ) in the ovaries than *C. gunnari* ( $0.1 \pm 0.0$ ) (Table 1).

339

### 340 3.4 Bioanalytical equivalents (BEQs)

341

342 The bioanalytical equivalents (BEQs) determined by DR-CALUX in the muscle of both  
343 species were in a similar range (*C. aceratus*:  $0.15 \pm 0.07$ , *C. gunnari*:  $0.2 \pm 0.14$  BEQ  $g^{-1}$  fw)  
344 to the  $WHO_{PCB}\text{-TEQs g}^{-1}$  fw, which were calculated using the concentrations of dioxin-like  
345 PCBs. In *C. gunnari*, however, the BEQs measured in the ovaries were about 70-fold higher  
346 (BEQ:  $5.14 \pm 3.6$ , TEQ:  $0.07 \pm 0.04$ ) than the calculated  $WHO_{PCB}\text{-TEQ g}^{-1}$  fw (Table 1).

347

348

## 349 4. Discussion

350

### 351 4.1 Species-specific contaminant patterns and levels

#### 352 4.1.1. Species-specific patterns

353

354 Literature data on present POP concentrations in tissues of Antarctic fish are scarce, and  
355 particularly those on contaminant levels in white-blooded icefish.

356 Earlier studies of the 80's and 90's measured PCB concentrations in the icefish *C. gunnari*  
357 and *C. aceratus* around the Antarctic Peninsula (Corsolini et al. 2002a, 2002b, 2009), which

358 were two to four times lower ( $0.7 \text{ ng g}^{-1}$  fw in muscle) than the ones of the present study.  
359 HCB levels of our icefish were within a similar range to those measured previously. It is  
360 known for sub-Antarctic fish that PCB levels can follow a seasonal trend, which is related to  
361 the release of pollutants from melting snow and ice during summer and the atmospheric  
362 transport of loads of pollutants which precipitate in Antarctic regions and are released during  
363 warming (Jaffal et al., 2011). Since sampling of our icefish took place in Austral autumn,  
364 after the seasonal ice melting in Antarctica, a seasonal release of POPs trapped in sea ice  
365 could thereby be one contributor to the comparably high levels of PCBs in our icefish  
366 samples. In fact, the PCB concentrations (per fw) of our icefish were within a similar range to  
367 those of salmon from the Baltic Sea (Isosaari et al., 2006).

368 In the early 00's, Borghesi et al. (2008; 2009) sampled *C. hamatus* and *C. gunnari* in the  
369 Ross Sea at  $74^\circ$  South. They found  $\Sigma$ PCB concentrations in *C. hamatus* muscle of  $0.35 \text{ ng g}^{-1}$   
370 fw,  $\Sigma$ non-ortho PCBs of  $5 \text{ ng g}^{-1}$  fw and  $\Sigma$ PBDEs  $0.16 \text{ ng g}^{-1}$  fw. In muscle of *C. gunnari*,  
371 they report  $\Sigma$ PBDEs of  $0.44 \text{ ng g}^{-1}$  fw. Those values are about five times higher than the  
372  $\Sigma$ PBDEs in muscle of *C. gunnari* measured in our study. Since they used fish of a similar  
373 size class, this difference is likely related to the measurement of different PBDE congeners in  
374 the studies.

375 Another recent study measured contaminant concentrations (in  $\text{ng g}^{-1}$  lw) in muscle, liver and  
376 gonads of three red-blooded Antarctic species, *Notothenia coriiceps*, *N. rossii* and  
377 *Trematomus newnesi*, from Potter Cove, Antarctic Peninsula (Lana et al., 2014). They  
378 reported similar  $\Sigma$ DDT values in muscle, but also species differences in the ovaries of their  
379 fish: while *N. coriiceps* and *T. newnesi* had similar values to *C. aceratus* measured in our  
380 study, *N. rossii* had much higher values than our fish. They also measured  $\gamma$ -HCH values  
381 about six-times higher than in our fish. *N. rossii* displays a rather benthic lifestyle, but also  
382 feeds on pelagic species, and thus has a prey spectrum similar to *C. gunnari*. Nevertheless,  
383 the DDTs and  $\gamma$ -HCH were much higher in *N. rossii* than in *C. gunnari*. In contrast,  $\Sigma$ PCB  
384 and  $\Sigma$ BDEs were highly variable among the species and tissues measured by Lana et al.  
385 (2014), but were generally within the same order of magnitude compared to our data. Thus,  
386 such strong differences in DDT and  $\gamma$ -HCH accumulation patterns between Antarctic fish  
387 species could also be related to a selective metabolism for individual contaminant classes  
388 between the species (Storelli et al., 2009), and not only to their ecological traits.

389

390 Amongst the PBDEs, BDE 47 showed the highest concentration (60% of all congeners) in  
391 our icefish amongst all PBDE congeners, followed by BDE 100 and BDE 99. This is in line

392 with the general picture of those congeners being the dominating PBDEs in fish around the  
393 globe, i.e. BDE 47 being recognized as the most important PBDE congener in marine biota  
394 (Zennegg et al., 2003; Isosaari et al., 2006; Kuiper et al., 2006).

395 In comparison to fish from non-Antarctic regions, PBDE concentrations on the fresh weight  
396 basis were particularly low in icefish. For example, PBDE concentrations range from about  
397 1.0 to 8 ng g<sup>-1</sup> fresh weight, or up to 64 ng g<sup>-1</sup> lipid weight in various fish species from the  
398 Baltic Sea (Isosaari et al., 2006), which are at least ten times higher than in the icefish.

399 Although the production of the former widely used brominated flame retardants penta- and  
400 octabromodiphenyl ether (PentaBDE and OctaBDE) were banned by the European Union in  
401 2004 and several states of the USA, toxic and persistent lower brominated PBDEs are still  
402 produced in other areas of the world and redistributed globally, also to the Antarctic (Cox and  
403 Efthymiou, 2003; Renner, 2004; Vives et al., 2004; Kuiper et al., 2006).

404 HCB concentrations (per fw) were also up to 30 times lower, and DDT concentrations (per  
405 fw) several hundreds of times lower in our icefish than in fish from the Northern hemisphere  
406 (Sharma et al., 2009). Despite a worldwide stop of the production of DDTs during the 70's, it  
407 has been reintroduced in the 2000s as malaria control by the WHO, and about 6000 tons of  
408 DDTs are still produced per year (UNEP, 2008). Due to its high persistence, bioaccumulation  
409 potential and cold condensation processes, DDTs and its metabolites are nowadays found in  
410 biota all over the world, and particularly in polar regions (Mirmigkou and de Boer, 2015).

411

412 The TEQs we calculated for *C. aceratus* and *C. gunnari* were in a similar range than TEQs  
413 reported for muscle of the icefish *C. hamatus* (TEQ 0.01-0.1 pg g<sup>-1</sup> wet weight) or red-  
414 blooded Antarctic fish (TEQ ~0.1 pg g<sup>-1</sup> wet weight) (Focardi et al.; Corsolini et al., 2002a;  
415 Borghesi et al., 2008). Generally, the values for Antarctic fish were lower than those for other  
416 organisms living in less remote parts of the world, e.g. WHO<sub>PCB</sub>-TEQ (pg g<sup>-1</sup> fw) of 3 to 15  
417 in muscle of salmon or Baltic herring. Yet, burbot from Bothnian bay (Baltic Sea) exhibit  
418 muscle TEQs, which are in a similar range as in Antarctic fish (Isosaari et al., 2006).

419 In *C. aceratus*, we found that the BEQs were comparable to the TEQs calculated on the basis  
420 on the WHO<sub>PCB</sub>-TEFs for salmonid fish species (Van den Berg et al., 1998). In comparison to  
421 fish from the northern hemisphere (Husain et al., 2014), Antarctic fish had about twenty  
422 times lower BEQ values in their muscle. In contrast, the BEQs in the ovaries of *C. gunnari*  
423 were much higher than the WHO<sub>PCB</sub>-TEQs calculated for their ovaries. The toxicity effects  
424 might thus be actually much higher than expected by the single usage of the calculated  
425 WHO<sub>PCB</sub>-TEQ. In fact, the BEQs in the ovaries of *C. gunnari* were within the same range as

426 BEQs of fish from temperate latitudes. Also Corsolini et al. (Corsolini et al., 2002a) stated  
427 that the TEQ values they measured in Antarctic fish were already half as high as those values  
428 which are considered to elicit toxicological effects, such as reproductive and immunological  
429 disorders, in marine mammals or birds (Kannan et al., 2000).

430 Rising PCB concentrations as observed by us in tissues of Antarctic fish will thus  
431 increasingly have the potential to exert their toxic effects on those fish.

432

#### 433 4.1.2. Temporal trends in contaminant levels

434

435 Long-term observations on contaminant levels in Antarctic biota are scarce. Yet, Weber and  
436 Goerke (2003; Goerke et al., 2004) measured contaminant levels in the liver of *C. aceratus*  
437 and *C. gunnari* in the same sampling area as in the present study. From 1987 to 1996, the  
438 authors measured an increase of PCB 153 and PCB 180 levels in *C. aceratus*, but not in *C.*  
439 *gunnari*. In the present study, we found about three times higher concentrations of those PCB  
440 congeners in the tissues of our two icefish species than in the previous study from 1996  
441 (Weber and Goerke, 2003). In contrast, the HCB concentrations show a declining trend from  
442 the 1987 study to our current survey. A similar trend of stable or declining HCB levels by up  
443 to 2.5% per year has also been observed in Arctic biota, such as birds, fish or marine  
444 mammals, since the late 80's (Barber et al., 2005; Rigét et al., 2010).

445

446 In liver of *C. aceratus*, concentrations of *p,p'*-DDE had already increased from 1987 to 1996,  
447 and the values we measured in muscle and ovaries of *C. aceratus* were almost twice as high  
448 as in 1996 (Weber and Goerke, 2003). In contrast, *p,p'*-DDE concentrations in *C. gunnari*  
449 remained at similar levels from 1987 over 1996 (study by Weber and Goerke (2003)) to the  
450 present study.

451 Nevertheless, overall DDT concentrations were increasing from 1987 to 1996 in both icefish  
452 species, and our values are also slightly higher than those in 1996, suggesting an increasing  
453 trend of DDTs in icefish around the Antarctic Peninsula. Despite a general global reduction  
454 of DDT as an insecticide, DDT is still produced at high volumes (see above, (UNEP, 2008)).  
455 Furthermore, climate warming on the one hand leads to an increased volatility and worldwide  
456 distribution of DDTs, and on the other hand local sources such as melting glaciers may  
457 additionally contribute to increasing DDT concentrations in tissues of Antarctic fish around  
458 the Antarctic Peninsula (van den Brink et al., 2009; van den Brink et al., 2011).

459

## 460 4.2 Correlation of contaminant concentrations with ecological traits

461

### 462 4.2.1 Tissue-specific patterns

463

464 Since all POPs analyzed in this study are highly lipophilic substances, tissue differences in  
465 the contaminant levels in *C. aceratus* and *C. gunnari* on fresh weight basis at first instance  
466 should correlate to tissue lipid concentrations (Corsolini et al., 2002a; Weber and Goerke,  
467 2003; Chiuchiolo et al., 2004). Indeed, *C. gunnari*, which possesses a clearly higher lipid  
468 content in the ovaries than *C. aceratus*, accordingly showed significantly higher PCB  
469 concentrations in its ovaries based on fresh weight compared to *C. gunnari*. Also HCB was  
470 two times more concentrated in the fat-rich ovaries of *C. aceratus* than in *C. gunnari*, but  
471 only on the lipid basis. Accordingly, the BEQs were higher in the ovaries of *C. aceratus* and  
472 *C. gunnari* than in their muscle tissue. Also Lana et al. (2014), investigating POP  
473 accumulation patterns in notothenioid species, reported that the highest levels were found in  
474 the gonads of the fish.

475

### 476 4.2.2 Ecological-related patterns

477

478 In addition to body lipid contents, our results point to an influence of habitat and trophic level  
479 on POP levels in white-blooded icefish. The benthic-living *C. aceratus* had two-times higher  
480 concentrations of almost all DDT congeners and HCB than the benthopelagic *C. gunnari*.  
481 Also the previous study by Weber and Goerke (2003) report higher (lipid-based) contaminant  
482 burdens in *C. aceratus* than in *C. gunnari* and highlight the higher tendency of *C. aceratus* to  
483 accumulate DDTs in its tissues than *C. gunnari* over the time.

484 Since POPs accumulate in sediments, benthic fish species are generally thought have a higher  
485 exposure and uptake of lipophilic contaminants (Goerke et al., 2004; Borghesi et al., 2008).

486 In addition to uptake from water or sediment, also the feeding habit thus plays a role, with  
487 species at higher trophic levels tending to show higher contaminant accumulation due to  
488 biomagnification. A recent study by Wolschke et al. (2015) also highlights the  
489 biomagnification of POPs from lower to higher trophic levels in the Antarctic food chain,  
490 which can be attributed to the diets of the animals.

491 From these observations, the benthic, piscivorous *C. aceratus* was expected to have higher

492 contaminant burdens than the benthopelagic, planktivorous *C. gunnari*. However, only HCB

493 and DDTs, but none of the other congener classes, were higher in the predominantly benthic  
494 *C. aceratus* than in the benthopelagic *C. gunnari* on the lipid weight basis.

495

496

## 497 **Conclusion**

498

499

500 Overall, PCB and DDT concentrations tend to rather increase than decrease in tissues of the  
501 two white-blooded icefish species *C. aceratus* and *C. gunnari* around Elephant Island and the  
502 South Shetland Islands, when compared to earlier studies. Our results thereby support the  
503 global transportation of POPs to the Southern Ocean and their bioaccumulation in the local  
504 marine fish, and point to a trend of increasing concentrations of POPs in Antarctic icefish.  
505 Our data also suggest that worldwide climate change effects may contribute to an increased  
506 volatilization and release of POPs trapped in glaciers, sea- or pack-ice, thereby leading to an  
507 ongoing contamination of the Southern Ocean and its biota.

508 Furthermore, we found differences in POP accumulation patterns between the two icefish  
509 species, which were weakly correlated to their trophic position. The piscivorous *C. aceratus*  
510 showed a higher potential to accumulate contaminants in its tissue than the planktivorous *C.*  
511 *gunnari*. This species difference highlights the influence of intake of POPs via the specific  
512 prey of individual fish species. However, the expected link between the contaminant burdens  
513 of *C. aceratus* and *C. gunnari* and their ecological traits could not be fully supported.

514 Additional factors, such species differences in toxicant metabolism rates and selective  
515 metabolism for single contaminant classes, may also play an important role in defining  
516 chemical bioaccumulation patterns in Antarctic fish species in the long term. In the end,  
517 Antarctic fish are a central link between the benthic community and top level predators,  
518 concomitantly POPs bioaccumulated in their tissues are likely to contribute to a progressive  
519 biomagnification of POPs along Antarctic food webs.

520

521

## 522 **Acknowledgements**

523 This work was funded by the Swiss National Science Foundation (SNSF 31003A\_149964/1)  
524 and the Freiwillige Akademische Gesellschaft Basel. We thank Donatella Perrone and  
525 Melanie Senn (Empa) for their helpful assistance in the laboratory.

526



527 **Tables & Figures**

528

529 **Tables**

530

531 **Table 1:** Lipid content (%) and levels of organic contaminants (ng g<sup>-1</sup> lipid weight; ng g<sup>-1</sup> fresh weight) (mean ± sem) in tissues of two Antarctic  
 532 icefish species.

533

	<i>C. aceratus</i> (n=10)				<i>C. gunnari</i> (n=11)					
Length (cm)	47-66				33-50					
Weight (g)	636-3620				252-888					
	lipid weight (ng g <sup>-1</sup> lw)		fresh weight (ng g <sup>-1</sup> fw)		lipid weight (ng g <sup>-1</sup> lw)		fresh weight (ng g <sup>-1</sup> fw)		Blank	
Tissue	muscle	ovaries	muscle	ovaries	muscle	ovaries	muscle	ovaries		
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
<b>TEQs</b>			<b>0.16</b>	<b>0.1</b>	<b>0.53</b>	<b>0.94</b>	<b>0.47</b>	<b>0.14*</b>	<b>0.07</b>	<b>0.04*</b>
<b>BEQs</b>			<b>0.15</b>	<b>0.07</b>	<b>0.57</b>	<b>0.75</b>	<b>0.20</b>	<b>0.14*</b>	<b>5.14</b>	<b>3.60*</b>

HCB	6.22	0.85 <sup>#</sup>	7.37	1.98 <sup>#</sup>	0.09	0.02	0.16	0.04	3.82	0.55 <sup>#</sup>	2.84	0.37 <sup>#</sup>	0.07	0.01 <sup>*</sup>	0.18	0.04 <sup>*</sup>	0.06
γ-HCH	0.58	0.21 <sup>*</sup>	1.18	0.49 <sup>*</sup>	0.01	<0.01 <sup>#,*</sup>	0.03	0.01 <sup>*</sup>	0.59	0.20	1.25	0.31	0.01	<0.01 <sup>#,*</sup>	0.07	0.02 <sup>*</sup>	0.07
<i>p,p'</i> -DDE	13.11	5.99	8.76	2.46 <sup>#</sup>	0.14	0.04 <sup>#,*</sup>	0.19	0.03 <sup>*</sup>	3.19	0.58	2.36	0.38 <sup>#</sup>	0.06	0.01 <sup>#,*</sup>	0.16	0.04 <sup>*</sup>	0.52
<i>o,p'</i> -DDT	3.20	0.98	2.83	0.65 <sup>#</sup>	0.04	0.01 <sup>*</sup>	0.07	0.01 <sup>*</sup>	1.65	0.36	1.64	0.38 <sup>#</sup>	0.03	<0.01 <sup>*</sup>	0.09	0.02 <sup>*</sup>	0.07
<i>p,p'</i> -DDT	5.31	1.61 <sup>#</sup>	6.21	1.10 <sup>#</sup>	0.06	0.02 <sup>*</sup>	0.15	0.02 <sup>*</sup>	2.19	0.42 <sup>#</sup>	2.98	0.52 <sup>#</sup>	0.04	0.01 <sup>*</sup>	0.19	0.04 <sup>*</sup>	0.32
<b>Σ DDTs</b>	<b>19.45</b>	<b>8.14<sup>#</sup></b>	<b>17.80</b>	<b>3.91<sup>#</sup></b>	<b>0.22</b>	<b>0.07</b>	<b>0.40</b>	<b>0.05</b>	<b>7.04</b>	<b>1.00<sup>#</sup></b>	<b>6.98</b>	<b>1.14<sup>#</sup></b>	<b>0.12</b>	<b>0.02<sup>*</sup></b>	<b>0.44</b>	<b>0.10<sup>*</sup></b>	
28	0.95	0.06 <sup>*</sup>	1.50	0.26 <sup>*</sup>	0.01	<0.01 <sup>*</sup>	0.04	0.01 <sup>#,*</sup>	1.23	0.11 <sup>*</sup>	2.84	0.53 <sup>*</sup>	0.04	0.01 <sup>*</sup>	0.18	0.05 <sup>#,*</sup>	0.12
52	2.15	0.79 <sup>*</sup>	3.29	0.98 <sup>*</sup>	0.02	0.01 <sup>*</sup>	0.09	0.03 <sup>*</sup>	1.56	0.38	7.72	2.31	0.36	0.34	0.37	0.09	0.25
101	5.12	1.48 <sup>*</sup>	7.10	1.69 <sup>*</sup>	0.06	0.01 <sup>*</sup>	0.19	0.05 <sup>#,*</sup>	3.99	0.97	10.74	2.72	0.68	0.63	0.60	0.12 <sup>#</sup>	0.26
138	5.74	1.21 <sup>*</sup>	8.20	1.83 <sup>*</sup>	0.07	0.01 <sup>*</sup>	0.22	0.05 <sup>*</sup>	5.47	1.23	11.06	2.69	0.60	0.52	0.68	0.18	0.40
153	6.00	1.26 <sup>*</sup>	8.72	2.10 <sup>*</sup>	0.07	0.01 <sup>*</sup>	0.23	0.06 <sup>*</sup>	5.94	1.40	11.96	2.52	0.41	0.33	0.76	0.20	0.49
180	1.94	0.31 <sup>*</sup>	3.06	0.67 <sup>*</sup>	0.02	<0.01 <sup>*</sup>	0.08	0.02 <sup>*</sup>	1.86	0.35 <sup>*</sup>	3.51	0.50 <sup>*</sup>	0.07	0.04	0.25	0.08	0.17
<b>Σ PCBs</b>	<b>21.91</b>	<b>4.98<sup>*</sup></b>	<b>31.87</b>	<b>7.26<sup>*</sup></b>	<b>0.26</b>	<b>0.03<sup>*</sup></b>	<b>0.85</b>	<b>0.21<sup>#,*</sup></b>	<b>20.04</b>	<b>4.27</b>	<b>47.82</b>	<b>10.24</b>	<b>2.17</b>	<b>1.86</b>	<b>2.85</b>	<b>0.63<sup>#</sup></b>	
28	0.21	0.09	0.83	0.37	<0.01	<0.01 <sup>*</sup>	0.02	0.01 <sup>*</sup>	0.22	0.08 <sup>*</sup>	0.87	0.33 <sup>*</sup>	<0.01	<0.01 <sup>*</sup>	0.05	0.02 <sup>*</sup>	<0.01

47	2.90	0.50*	5.32	1.10*	0.04	<0.01*	0.15	0.03*	2.46	0.39*	10.14	1.41*	0.04	0.01*	0.70	0.26*	0.33
100	0.59	0.12*	1.27	0.27*	0.01	<0.01*	0.04	0.01*	0.45	0.10*	2.17	0.42*	0.01	<0.01*	0.15	0.05*	0.11
99	0.99	0.47*	1.97	0.49 <sup>#,*</sup>	0.01	0.02*	0.06	0.02*	0.86	0.12*	5.28	1.01 <sup>#,*</sup>	0.02	<0.01*	0.39	0.19*	0.05
153	0.11	0.02	0.21	0.07	<0.01	<0.01*	0.01	<0.01*	0.07	0.01*	0.53	0.09*	<0.01	<0.01*	0.04	0.02*	0.01.
183	0.08	0.01 <sup>#</sup>	0.16	0.07	<0.01	<0.01 <sup>#,*</sup>	<0.01	<0.01 <sup>#,*</sup>	0.04	0.01 <sup>#</sup>	0.53	0.09	<0.01	<0.01 <sup>#,*</sup>	0.04	0.02 <sup>#,*</sup>	0.01
197	0.13	0.03 <sup>#,*</sup>	0.27	0.08 <sup>#,*</sup>	<0.01	<0.01*	0.01	<0.01 <sup>#,*</sup>	0.07	0.01 <sup>#,*</sup>	0.33	0.10 <sup>#,*</sup>	<0.01	<0.01*	0.02	0.01 <sup>#,*</sup>	0.01
<b>Σ PBDEs</b>	<b>5.01</b>	<b>0.82*</b>	<b>10.03</b>	<b>1.86*</b>	<b>0.07</b>	<b>0.01*</b>	<b>0.29</b>	<b>0.06*</b>	<b>4.08</b>	<b>0.54*</b>	<b>0.72</b>	<b>0.14*</b>	<b>0.08</b>	<b>0.01*</b>	<b>0.05</b>	<b>0.02*</b>	
%lipid					1.45	0.18	2.64	0.23 <sup>#</sup>					2.13	0.35*	6.93	1.32 <sup>#,*</sup>	

534 In **bold**: sum (Σ) of all DDT, PCB & PBDE congeners. Lw: lipid weight, fw: fresh weight. TEQ: toxic equivalents, pg WHO-TEQ<sup>-1</sup>g fw,  
535 calculated by using the toxic equivalency factors recommended by Van den Berg (1998). BEQ: bioequivalent values, BEQ g<sup>-1</sup> fw. The # denotes a  
536 significant difference between *C. gunnari* and *C. aceratus* in the given tissue at  $p \leq 0.05$ . The \* denotes a significant difference between tissues for  
537 each species at  $p \leq 0.05$ .

538 **Figure Captions**

539

540 **Figure 1** Mean ( $\pm$  sem) concentration of  $\Sigma$  PCBs,  $\Sigma$  BDEs, HCBs,  $\gamma$ -HCHs and  $\Sigma$  DDTs (ng  
541  $\text{g}^{-1}$  lipid weight), in muscle and ovaries of the two icefish species, *C. gunnari* ( $n=11$ ) and *C.*  
542 *aceratus* ( $n=10$ ). The # denotes a significant difference between *C. gunnari* and *C. aceratus* in  
543 the given tissue at  $p \leq 0.05$ . The \* denotes a significant difference between tissues for each  
544 species at  $p \leq 0.05$ .

545

546 **Figure 2:** Congener composition (percentage) of PCBs, DDTs and BDEs in muscle (m) and  
547 ovaries (ov) of *C. gunnari* (Cg) and *C. aceratus* (Ca).

548

549

551 **References**

- 552 Barber, J.L., Sweetman, A.J., Van Wijk, D., Jones, K.C., 2005. Hexachlorobenzene in the  
 553 global environment: emissions, levels, distribution, trends and processes. *Sci. Total. Environ.*  
 554 349, 1-44.
- 555 Bargagli, R., 2008. Environmental contamination in Antarctic ecosystems. *Sci. Total.*  
 556 *Environ.* 400, 212-226.
- 557 Bogdal, C., Nriagu, D., Luthy, M.P., Schenker, S., Scheringer, M., Hungerbühler, K., 2010.  
 558 Release of legacy pollutants from melting glaciers: model evidence and conceptual  
 559 understanding. *Environ. Sci. Technol.* 44, 4063-4069.
- 560 Borghesi, N., Corsolini, S., Focardi, S., 2008. Levels of polybrominated diphenyl ethers  
 561 (PBDEs) and organochlorine pollutants in two species of Antarctic fish (*Chionodraco*  
 562 *hamatus* and *Trematomus bernacchii*). *Chemosphere* 73, 155-160.
- 563 Borghesi, N., Corsolini, S., Leonards, P., Brandsma, S., de Boer, J., Focardi, S., 2009.  
 564 Polybrominated diphenyl ether contamination levels in fish from the Antarctic and the  
 565 Mediterranean Sea. *Chemosphere* 77, 693-698.
- 566 Cabrerizo, A., Dachs, G., Barcel, D., Jones, K.C., 2013. Climatic and biogeochemical  
 567 controls on the remobilization and reservoirs of persistent organic pollutants in Antarctica.  
 568 *Environ. Sci. Technol.* 47, 4299-4306.
- 569 Chiuchiolo, A.L., Dickhut, R.M., Cochran, M.A., Ducklow, H.W., 2004. Persistent organic  
 570 pollutants at the base of the Antarctic marine food web. *Environ. Sci. Technol.* 38, 3551-  
 571 3557.
- 572 Corsolini, S., Borghesi, N., Schiamone, A., Focardi, S., 2007. Polybrominated diphenyl  
 573 ethers, polychlorinated dibenzo-dioxins,-furans, and-biphenyls in three species of Antarctic  
 574 penguins. *Environ. Sci. Pollut. R.* 14, 421-429.
- 575 Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., Giesy, J.P., 2002a.  
 576 Polychloronaphthalenes and other dioxin-like compounds in Arctic and Antarctic marine food  
 577 webs. *Environ. Sci. Technol.* 36, 3490-3496.
- 578 Corsolini, S., Ademollo, N., Mariottini, M., Fossi, S., Guerranti, C., Perra, G., Duhamel, G.,  
 579 Focardi, S., 2002b. Polychlorinated biphenyls, polychlorinated-dibenzodioxins and –  
 580 dibenzofurans in mackerel icefish and marbled rockcod from the Kerguelen Islands  
 581 (Antarctica). *Food and Feed I*, 57, 161 – 164.
- 582 Cox, P., Efthymiou, P., 2003. Directive 2003/11/EC of the European parliament and of the  
 583 council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC  
 584 relating to restrictions on the marketing and use of certain dangerous substances and  
 585 preparations (pentabromodiphenyl ether, octabromodiphenyl ether). *Off. J. Eur. Union* 42, 45-  
 586 46.
- 587 Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for  
 588 upwelling of corrosive "acidified" water onto the continental shelf. *Science* 320, 1490-1492.
- 589 Focardi, S., Fossi, M., Lari, L., Casini, S., Leonzio, C., Meidel, S., Nigro, M., 1995. Induction  
 590 of MFO activity in the antarctic fish *Pagothenia bernacchii*: Preliminary results. *Mar.*  
 591 *Environ. Res.* 39, 97-100.
- 592 Goerke, H., Weber, K., Bornemann, H., Ramdohr, S., Plötz, J., 2004. Increasing levels and  
 593 biomagnification of persistent organic pollutants (POPs) in Antarctic biota. *Mar. Pollut. Bull.*  
 594 48, 295-302.
- 595 Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G., 2013.  
 596 Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica.  
 597 *Mar. Pollut. Bull.* 77, 82-89.

598 Hale, R.C., Kim, S.L., Harvey, E., La Guardia, M.J., Mainor, T.M., Bush, E.O., Jacobs, E.M.,  
599 2008. Antarctic research bases: local sources of polybrominated diphenyl ether (PBDE) flame  
600 retardants. *Environ. Sci. Technol.* 42, 1452-1457.

601 Hartmann, R., 2013. Extraction of PCDD/Fs and PCBs in fish using SpeedExtractor E-914.  
602 *Proc. Environ. Sci.* 18, 875-881.

603 Husain, A., Gevao, B., Dashti, B., Brouwer, A., Behnisch, P.A., Al-Wadi, M., Al-Foudari,  
604 M., 2014. Screening for PCDD/Fs and dl-PCBs in local and imported food and feed products  
605 available across the State of Kuwait and assessment of dietary intake. *Ecotox. Environ. Safe.*  
606 100, 27-31.

607 Isoaari, P., Hallikainen, A., Kiviranta, H., Vuorinen, P.J., Parmanne, R., Koistinen, J.,  
608 Vartiainen, T., 2006. Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls,  
609 naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea  
610 and lakes in Finland. *Environ. Pollut.* 141, 213-225.

611 Jaffal, A., Paris-Palacios, S., Jolly, S., Thailly, A., Delahaut, L., Beall, E., Roche, H.,  
612 Biagianti-Risbourg, S., Betoulle, S., 2011. Cadmium and copper contents in a freshwater fish  
613 species (brook trout, *Salvelinus fontinalis*) from the subantarctic Kerguelen Islands. *Polar*  
614 *Biol.* 34, 397-409.

615 Kannan, K., Blankenship, A., Jones, P., Giesy, J., 2000. Toxicity reference values for the  
616 toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum. Ecol. Risk Assess.* 6,  
617 181-201.

618 Kohler, M., Zennegg, M., Bogdal, C., Gerecke, A.C., Schmid, P., V. Heeb, N., Sturm, M.,  
619 Vonmont, H., E. Kohler, H.-P., Giger, W., 2008. Temporal trends, congener patterns, and  
620 sources of octa-, nona-, and decabromodiphenyl ethers (PBDE) and  
621 hexabromocyclododecanes (HBCD) in Swiss lake sediments. *Environ. Sci. Technol.* 42,  
622 6378-6384.

623 Kuiper, R.V., Murk, A., Leonards, P.E., Grinwis, G., Van den Berg, M., Vos, J.G., 2006. In  
624 vivo and in vitro Ah-receptor activation by commercial and fractionated  
625 pentabromodiphenylether using zebrafish (*Danio rerio*) and the DR-CALUX assay. *Aquat.*  
626 *Toxicol.* 79, 366-375.

627 Lana, N.B., Berton, P., Covaci, A., Ciocco, N.F., Barrera-Oro, E., Atencio, A., Altamirano,  
628 J.C., 2014. Fingerprint of persistent organic pollutants in tissues of Antarctic notothenioid  
629 fish. *Sci. Total Environ.* 499, 89-98.

630 Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault,  
631 J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent  
632 organohalogen contaminants in arctic wildlife and fish. *Sci. Total Environ.* 408, 2995-3043.

633 Mintenbeck, K., Barrera-Oro, E.R., Brey, T., Jacob, U., Knust, R., Mark, F.C., Moreira, E.,  
634 Strobel, A., Arntz, W.E., 2012. 5 - Impact of Climate Change on Fishes in Complex Antarctic  
635 Ecosystems. in: Ute, J., Guy, W. (Eds.). *Adv. Ecol. Res.* Academic Press, 351-426.

636 Mirmigkou, S., de Boer, J., 2015. DDT and Metabolites. *The Handbook of Environmental*  
637 *Chemistry*, 1-24.

638 Murk, A., Legler, J., Denison, M., Giesy, J., Van de Guchte, C., Brouwer, A., 1996.  
639 Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah  
640 receptor active compounds in sediments and pore water. *Toxicol. Sci.* 33, 149-160.

641 Nash, S.B., 2011. Persistent organic pollutants in Antarctica: current and future research  
642 priorities. *J. Environ. Monitor.* 13, 497-504.

643 Nichols, J.W., Huggett, D.B., Arnot, J.A., Fitzsimmons, P.N., Cowan - Ellsberry, C.E., 2013.  
644 Toward improved models for predicting bioconcentration of well - metabolized compounds  
645 by rainbow trout using measured rates of in vitro intrinsic clearance. *Environmental*  
646 *Toxicology and Chemistry* 32, 1611-1622.

647 Renner, R., 2004. Small fish for healthy babies. *Environ. Sci. Technol.* 38, 262-263.

648 Schmid, P., Bogdal, C., Bl t hgen, N., Anselmetti, .S., wyssig, A., Hungerb hle r, K., 2010.  
649 The Missing Piece: Sediment Records in Remote Mountain Lakes Confirm Glaciers Being  
650 Secondary Sources of Persistent Organic Pollutants. *Environ. Sci. Technol.* 45, 203-208.  
651 Schmid, P., Kohler, M., Gujer, E., Zennegg, M., Lanfranchi, M., 2007. Persistent organic  
652 pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes  
653 in Switzerland. *Chemosphere* 67, 16-21.  
654 Sharma, C.M., Rosseland, B.O., Almvik, M., Eklo, O.M., 2009. Bioaccumulation of  
655 organochlorine pollutants in the fish community in Lake Årungen, Norway. *Environ. Pollut.*  
656 157, 2452-2458.  
657 Simonich, S.L., Hites, R.A., 1995. Global distribution of persistent organochlorine  
658 compounds. *Science* 269, 1851.  
659 Rigét, F., Bignert, A., Braune, B., Stow, J., Wilson, S., 2010. Temporal trends of legacy POPs  
660 in Arctic biota, an update. *Sci. Total Environ.* 408, 2874-2884.  
661 Storelli, M., Losada, S., Marcotrigiano, G., Roosens, L., Barone, G., Neels, H., Covaci, A.,  
662 2009. Polychlorinated biphenyl and organochlorine pesticide contamination signatures in  
663 deep-sea fish from the Mediterranean Sea. *Environ. Res.* 109, 851-856.  
664 Strobel, A., Burkhardt-Holm, P., Schmid, P., Segner, H., 2015. Benzo (a) pyrene metabolism  
665 and EROD and GST biotransformation activity in the liver of red-and white-blooded  
666 Antarctic fish. *Environ. Sci. Technol.* 49, 8022-8032.  
667 UNEP, 2002. Antarctica Regional Report. In: Chemicals, U.N.E.P. (Ed.). Regionally Based  
668 Assessment Of Persistent Toxic Substances.  
669 UNEP, 2008. Global Status Of DDT And Its Alternatives For Use In Vector Control To  
670 Prevent Disease. In: UNEP/POPS/DDTBP.1/2 (Ed.). Stockholm Convention on Persistent  
671 Organic Pollutants. UNEP, Geneva.  
672 UNEP/AMAP, 2011. Climate Change and POPs: Predicting The Impacts. Report of the  
673 UNEP/AMAP Expert Group. Geneva.  
674 Van den Berg, M., Birnbaum, L., Bosveld, A., Brunström, B., Cook, P., Feeley, M., Giesy,  
675 J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., 1998. Toxic equivalency factors (TEFs) for  
676 PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Persp.* 106, 775.  
677 van den Brink, N., Riddle, M., Heuvel-Greve, M.v.d., Allison, I., Snape, I., van Franeker,  
678 J.A., 2009. Correspondence on Geisz et al. Melting glaciers: a probable source of DDT to the  
679 Antarctic marine ecosystem. *Environ. Sci. Technol.* 43, 3976-3977.  
680 van den Brink, N.W., Riddle, M.J., van den Heuvel-Greve, M., van Franeker, J.A., 2011.  
681 Contrasting time trends of organic contaminants in Antarctic pelagic and benthic food webs.  
682 *Mar. Pollut. Bull.* 62, 128-132.  
683 Vives, I., Grimalt, J.O., Fernandez, P., Rosseland, B., 2004. Polycyclic aromatic  
684 hydrocarbons in fish from remote and high mountain lakes in Europe and Greenland. *Sci.*  
685 *Total Environ.* 324, 67-77.  
686 Wania, F., Mackay, D., 1993. Global fractionation and cold condensation of low volatility  
687 organochlorine compounds in polar regions. *Ambio*, 10-18.  
688 Weber, K., Goerke, H., 2003. Persistent organic pollutants (POPs) in Antarctic fish: levels,  
689 patterns, changes. *Chemosphere* 53, 667-678.  
690 Wolschke, H., Meng, X.-Z., Xie, Z., Ebinghaus, R., Cai, M., 2015. Novel flame retardants (N-  
691 FRs), polybrominated diphenyl ethers (PBDEs) and dioxin-like polychlorinated biphenyls  
692 (DL-PCBs) in fish, penguin, and skua from King George Island, Antarctica. *Mar. Pollut. Bull.*  
693 96, 513-518.  
694 Xie, ., M ller, A., Ahrens, L., Sturm, R., Ebinghaus, R., 2011. Brominated flame retardants  
695 in seawater and atmosphere of the Atlantic and the Southern Ocean. *Environ. Sci. Technol.*  
696 45, 1820-1826.  
697 Zennegg, M., Kohler, M., Gerecke, A.C., Schmid, P., 2003. Polybrominated diphenyl ethers  
698 in whitefish from Swiss lakes and farmed rainbow trout. *Chemosphere* 51, 545-553.

**Manuscript No. CHEM38908**

**Highlights**

- PCB and DDT concentrations in icefish are higher than those measured in the late 90s
- Mature, female icefish possess higher contaminant levels in ovaries than in muscle
- POP levels are similar in fish from different sampling sites around the Antarctic Peninsula
- Piscivorous icefish display a higher toxicant accumulation than planktivorous icefish
- Bioanalytical results (BEQs) were similar to chemical analytical calculations (TEQs) in icefish





## 25 **Abstract**

26

27 The global occurrence of persistent organic pollutants (POPs) continuously contributes to  
28 their accumulation also in remote areas such as the Antarctic Ocean. Antarctic fish, which  
29 hold high trophic positions but appear to possess low endogenous elimination rates for  
30 chemicals, are expected to bioaccumulate POPs with rising anthropogenic pollution.  
31 Using a chemical-analytical method, we measured concentrations of PCBs, PBDEs, HCBs,  
32 HCH and DDTs and determined toxic equivalents (TEQs) and bioanalytical equivalents  
33 (BEQs) in muscle and ovaries of Antarctic icefish caught in the Southern Ocean around  
34 Elephant Island. We used two species with different feeding habits and trophic web positions:  
35 the planktivorous *Champsocephalus gunnari* and the piscivorous *Chaenocephalus aceratus*.  
36 Our results revealed higher contaminant levels in ovary than in muscle tissues of both  
37 species. Most analytes concentrations and the TEQs (0.2-0.5) and BEQs (0.2) were lower as  
38 in temperate species. Comparison with literature data points to higher PCB (20-22 ng g<sup>-1</sup> lipid  
39 weight (lw)) and DDT (7-19.5 ng g<sup>-1</sup> lw) concentrations than those measured in icefish in the  
40 90's. For the other contaminants, we could not identify temporal trends. We found a higher  
41 bioaccumulation of contaminants, particularly HCB and DDTs, in *C. aceratus* (6.2 & 19.5 ng  
42 g<sup>-1</sup> lw, respectively) than in *C. gunnari* (3.8 & 7.0 ng g<sup>-1</sup> lw, respectively). However, there  
43 was no general species-specific accumulation pattern of the different toxicant classes between  
44 the two icefish. Thus, the expected link between contaminant burdens of *C. aceratus* and *C.*  
45 *gunnari* and their ecological traits was only weakly supported for these species.

46

## 47 **Highlights**

48

- 49 - PCB and DDT concentrations in icefish are higher than those measured in the late 90s
- 50 - Mature, female icefish possess higher contaminant levels in ovaries than in muscle
- 51 - POP levels are similar in fish from different sampling sites around the Antarctic  
52 Peninsula
- 53 - Piscivorous icefish display a higher toxicant accumulation than planktivorous icefish
- 54 - Bioanalytical results (BEQs) were similar to chemical analytical calculations (TEQs)  
55 in icefish

56

## 57 **Keywords**

58 Icefish, bioaccumulation, persistent organic pollutants, polychlorinated biphenyls (PCBs),  
59 toxic equivalents (TEQs), DR CALUX bioanalytical equivalents (BEQs)

60  
61

## 62 **1. Introduction**

63

64 Antarctica has been less affected by human influences than other continents for a long time,  
65 however, contamination with anthropogenic contaminants, in particularly persistent organic  
66 pollutants (POPs) has increased progressively (Nash, 2011; UNEP/AMAP, 2011). Nowadays,  
67 Antarctica serves as a major sink for highly persistent contaminants. Long-range atmospheric  
68 transport, together with global distillation processes and cold condensation, are considered to  
69 be the main mechanisms for the progressive contamination of the Antarctic ecosystem,  
70 together with local sources such as fishing, tourism and research activities (Simonich and  
71 Hites; Feely et al., 2008; Nash, 2011). Particularly POPs such as polychlorinated biphenyls  
72 (PCBs) and polybrominated diphenyl ethers (PBDEs), and amongst them also the formerly  
73 used insecticides such as  $\gamma$ -hexachlorocyclohexane ( $\gamma$ -HCH) and *p,p'*-DDT are ubiquitous  
74 pollutants with a wide application spectrum. For example, polymer additives such as flame  
75 retardants are found globally in building material, furniture, paint, textiles or plastics, which  
76 are also used in Antarctic bases and vessels cruising in these regions (Hale et al., 2008;  
77 Kohler et al., 2008). This worldwide abundance and the high persistence of POPs  
78 continuously contribute to an accumulation in the ice masses and biota of polar regions  
79 (Wania and Mackay, 1993; Chiuchiolo et al., 2004; Goerke et al., 2004; Corsolini et al.,  
80 2007; Bargagli, 2008; Borghesi et al., 2008; Borghesi et al., 2009; Xie et al., 2011; Wolschke  
81 et al., 2015).

82

83 Furthermore, those lipophilic organic chemicals have a high potential to bioaccumulate in  
84 aquatic biota, and particularly in Antarctic species, which generally possess low endogenous  
85 elimination rates for those chemicals (Strobel et al., 2015). Additionally, it is expected that  
86 climate warming will lead to the release of those pollutants trapped in glaciers and sea-ice,  
87 which additionally contributes to increasing POP levels in the tissues of Antarctic animals  
88 (Weber and Goerke, 2003; Bogdal et al., 2010; Schmid et al., 2010; van den Brink et al.,  
89 2011; Cabrerizo et al., 2013; Goutte et al., 2013). As global chemical usage continues to  
90 grow, including the usage of persistent and bioaccumulative compounds, it is to be expected  
91 that contaminant intake into Antarctica will further increase in the future.

92

93 The endemic, Antarctic notothenioid fish are evolutionary well-adapted to the cold and stable  
94 environment of the Southern Ocean. Adaptations involve e.g. high amounts of tissue lipids,  
95 slow growth rates, long life spans and slow metabolism and elimination rates for xenobiotics  
96 (Mintenbeck et al., 2012; Strobel et al., 2015). All these factors may favor the  
97 bioaccumulation of lipophilic contaminants in the tissues of those fish.

98

99 Lipophilic, non-charged contaminants like PCBs can be taken up via a physico-chemically  
100 driven, passive partitioning of the chemicals from the water phase into the lipid phase of the  
101 organism and thereby accumulate differentially in tissues or fish species, depending on their  
102 lipophilicity of the chemical and the lipid content or composition of the tissues (Nichols et  
103 al., 2013). In fact, tissue-specific differences of POP levels are reported for notothenioid  
104 species (Lana et al., 2014). Furthermore, bioaccumulation and biomagnification can be  
105 related to an organism's habitat and its trophic position within the food web (Corsolini et al.,  
106 2002a; Weber and Goerke, 2003; Chiuchiolo et al., 2004). Particularly for highly lipophilic  
107 compounds, oral uptake via the prey contributes by a major extent to the bioaccumulation of  
108 toxicants in the tissues of a species. For example, Weber and Goerke (2003) found higher  
109 contaminant levels in the piscivorous icefish *Chaenocephalus aceratus* than in the  
110 planktivorous icefish *Champscephalus gunnari*, which was apparently related to the  
111 different food spectra of the two notothenioids (Goerke et al., 2004). Such studies highlight  
112 that POPs are transferred within the Antarctic food web, leading to increasing POP  
113 concentrations along the food chain up to high concentrations in top level predators  
114 (Wolschke et al., 2015).

115

116 Considering the trends of rising POP concentrations in Antarctica (UNEP/AMAP, 2011 and  
117 their high potential to exert toxic effects in marine biota (Nash, 2011), it is very important to  
118 keep monitoring body burdens of the fish living in the Southern Ocean. Yet, we are far from  
119 having a solid understanding of the contamination status, time trends, the diversity of  
120 contaminants or their toxicity potential in Antarctic biota (UNEP, 2002; UNEP/AMAP,  
121 2011). Particularly the bioaccumulation pattern of contaminants such as the polybrominated  
122 flame retardants (PBDEs) or insecticides (e.g. DDT) have hardly been measured in icefish.

123

124 The aim of this study was to determine levels of selected POPs in muscle and ovary tissue of  
125 two white-blooded Antarctic notothenioid species in the study area around Elephant Island,

126 the South Shetland Islands and the Antarctic Peninsula, and to estimate temporal trends of  
127 fish POP levels in this area. The analytically measured contaminant concentrations were  
128 converted into toxic equivalents (TEQs) using standardized values. In order to examine  
129 whether food web position or tissue lipid content has an influence on chemical  
130 concentrations, we analyzed lipid content and POP levels in fish species of the same family  
131 but with different feeding habits and trophic web positions, namely the planktivorous *C.*  
132 *gunnari* and the piscivorous *C. aceratus*.  
133 Extending the classical contaminants, this study includes polybrominated flame retardants  
134 (PBDEs) and insecticides (i.e. DDT, HCB). In addition to chemical analytics, we also applied  
135 bioanalytics using the DR-CALUX assay (Murk et al., 1996; Kuiper et al., 2006) to assess the  
136 total accumulated dioxin-like activity in the fish tissues.

137

138

## 139 **2. Methods**

140

### 141 2.1 Study species

142 Two species of the *Channichthyidae* (white-blooded icefish) were fished with bottom trawls  
143 down to 500 m, during a cruise with the research vessel 'Polarstern' (ANTXXVIII/4, March  
144 13 to April 9, 2012; [http://expedition.awi.de/expedition/ANT-](http://expedition.awi.de/expedition/ANT-XXVIII/4?alias=PS79#mapChart)  
145 [XXVIII/4?alias=PS79#mapChart](http://expedition.awi.de/expedition/ANT-XXVIII/4?alias=PS79#mapChart)) at different, closely located sampling sites around Elephant  
146 Island and the South Shetland Islands (61.1°S, 55.1°W). Only fish netted alive and without  
147 macroscopically visible damage were used for tissue sampling. The planktivorous mackerel  
148 icefish, *C. gunnari*, shows a mainly benthopelagic feeding mode, while the piscivorous  
149 Scotia Sea icefish *C. aceratus* is predominantly a benthos feeder (Weber and Goerke, 2003).  
150 Persistent organic pollutants were analyzed in muscle and ovary tissue of mature (stage III-  
151 IV), female fish only. Sex and maturity stage of the fish were verified histologically. Animal  
152 weight and length are given in Table 1. All tissue samples were wrapped in aluminum foil  
153 and stored immediately at -20 °C until used for analysis.

154

### 155 2.2 Sample preparation

156

157 Muscle and gonad samples were defrosted, cut into pieces and lyophilized at 33 Pa for 72  
158 hours until constant weight. Dried tissue of muscle (5-10 g) or gonads (0.5-2 g) was ground

159 with anhydrous sodium sulfate and quartz sand in a ceramic mortar and pestle to obtain a fine  
160 powder.

161 This homogenate was then Soxhlet-extracted with a speed-extractor (E-914, Büchi,  
162 Switzerland) in 120 mL extraction cells at a constant temperature and pressure of 100 °C and  
163 100 bar, respectively (4 cycles, hold time 10 min., discharge 4 min), with ~150 ml *n*-  
164 hexane/dichloromethane 1:1 (v:v) (Hartmann, 2013). The extract was concentrated in a  
165 Syncore evaporator (Büchi, Switzerland) and let dry completely by applying a gentle  
166 nitrogen stream. The residue accounted for the fat content of the sample. <sup>13</sup>C<sub>12</sub> labeled  
167 internal standards (Schmid et al., 2007) were added to the samples and after the addition of 2  
168 – 3 mL of *n*-hexane the solution was treated with 3 ml oleum (7% SO<sub>3</sub> in conc. sulfuric acid).  
169 After centrifugation for 3 min at 5'000 rpm, the solvent layer with the lipophilic target  
170 analytes was removed and the remaining suspension was re-extracted two times more with *n*-  
171 hexane. The pooled extracts were concentrated to 0.5 mL in a rotary evaporator at 45 °C and  
172 300 mbar. Subsequently, the extract was purified on a multilayer mini silica gel column (from  
173 top to bottom: 0.25 g anhydrous sodium sulfate, 0.25 g silica gel 60 with 44% sulfuric acid  
174 and 0.25 g silica gel 60 activated at 130 °C). The sample was applied on the column and  
175 eluted with 5 ml *n*-hexane, followed by 5 mL *n*-hexane/dichloromethane 1:1 (v:v). The eluate  
176 was concentrated using a rotary evaporator to 0.5 mL. After transfer to a mini GC-Vial the  
177 volume was further reduced to 30 µL by the application of a gentle stream of nitrogen at  
178 room temperature. Finally the recovery standard <sup>13</sup>C<sub>12</sub> labeled PCB 70 was added. Samples  
179 were stored in toluene at -20 °C until analysis. Method blank levels for the whole analytical  
180 procedure were determined in duplicates (Table 1 and A.2).

181

### 182 2.3 Chemical analysis

183

184 PCBs (indicator PCB 28, 52, 101, 138, 153, and 180; dioxin-like PCBs 77, 81, 105, 114, 118,  
185 123, 126 156, 157, 167, 169, and 189), DDT (*o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE),  
186 hexachlorobenzene (HCB),  $\gamma$ -hexachlorocyclohexane ( $\gamma$ -HCH), and PBDEs (BDE 28, 47, 99,  
187 100, 153, 154, 183, and 209) were included in this study. Quantitative determination of the  
188 target analytes in the extracts was achieved by gas chromatography/high resolution mass  
189 spectrometry (GC/HRMS). Analyses were carried out on a Finnigan MAT95 high-resolution  
190 mass spectrometer (Thermo Finnigan MAT, Bremen Germany) coupled to a Finnigan Trace

191 GC Ultra equipped with a Triplus auto sampler (Thermo Electron Corporation, Waltham,  
192 MA, USA). Samples were injected in splitless mode (splitless time 30 s) at an injector  
193 temperature of 260 °C. For the gas chromatographic separation a RTX5 Sil-MS column (30  
194 m × 0.25 mm, film thickness 0.10 µm) was used with helium as carrier gas at a pressure of  
195 100 kPa. The following temperature programs were used for the different compound classes.  
196 For the PCBs, the initial column temperature was 100 °C. After 0.5 min, the temperature was  
197 ramped at 20 °C/ min to 180 °C, followed by 3 °C/ min to 250 °C, and 20 °C/ min to 300 °C.  
198 For pesticides, the initial column temperature was 100 °C. After 0.5 min, the temperature was  
199 ramped at 10 °C/ min to 160 °C, followed by 4 °C/ min to 240 °C, and 20 °C/ min to 300 °C.  
200 For the PBDEs, the initial column temperature was 100 °C. After 0.5 min, the temperature  
201 was ramped at 20 °C/ min to 220 °C, followed by 6 °C/ min to 300 °C, and 10 °C/ min to 320  
202 °C. The ion source was operated at 220 °C, the electron energy was 70eV, and the mass  
203 spectrometer was tuned to a mass resolution of 8000-10000. The two most abundant signals  
204 of the molecular ion cluster of the analytes and the <sup>13</sup>C<sub>12</sub> labeled internal standards were  
205 recorded in the single ion monitoring mode.

206

207 The analytes were identified by comparing the retention times with those of the labeled  
208 internal standards. Quantification was based on peak areas of the analytes and the labeled  
209 reference compounds with known concentrations. More details about the method are  
210 available in the literature (Zennegg et al., 2003; Schmid et al., 2007).

211 For the calculation of TEQs, the dioxin-like PCBs were determined in the same way, but as  
212 part of the sample extract was used later on for the analysis by the bioassay (DR-CALUX),  
213 no <sup>13</sup>C<sub>12</sub>-labeled internal dl-PCBs standards could be used for the quantification, as the  
214 isotope labeled analogues exhibit similar activity in the DR-CALUX. Therefore, quantitative  
215 determination of dl-PCBs was based on the <sup>13</sup>C<sub>12</sub> labeled indicator PCBs used as internal  
216 standards and previously determined response factors to native dl-PCBs.

217 The blank values (in ng g<sup>-1</sup> lipid weight) were all below the compound concentrations and are  
218 given in Table 1 and A.1.

219 The limit of detection (LOD) and the limit of quantification (LOQ) were set by definition at  
220 signal to noise ratios of greater than three (s/n ≥ 3) and ten (s/n ≥ 10) respectively. All glass  
221 ware used were cleaned with strongly alkaline detergents and backed out overnight in a  
222 ceramic oven at 450 °C. Directly before use, the glass ware was rinsed with solvents (*n*-  
223 hexane, dichloromethane).

224

## 225 2.4 Determination of dioxin-like toxic equivalents (TEQs)

226

227 In order to assess total dioxin-like activity in the fish tissues, we calculated the 2,3,7,8-  
228 tetrachlorodibenzo-*p*-dioxin (TCDD) equivalent (TEQ) concentrations of dl-PCBs in the  
229 muscle and gonad extracts of both icefish species using Toxic Equivalency Factors (TEFs)  
230 proposed by the World Health Organization (WHO) for fish (Van den Berg et al., 1998).  
231 TEQs were calculated as the sum of the TEFs of all dl-PCBs listed in Table A.1. Although  
232 the TEF values are not derived from studies with Antarctic fish, since TEFs are only available  
233 for salmonids (Van den Berg et al., 1998) they provide a useful tool for a reasonable estimate  
234 of toxicity effects of PCBs on Antarctic fish.

235

## 236 2.5 Determination of bioequivalent values (BEQs)

237

238 The same extracts that were used for chemical analysis were also used in a standard bioassay,  
239 the DR-CALUX (Dioxin Responsive Chemically Activated Luciferase Gene Expression)  
240 assay. This cell and receptor based reporter gene bioassay measures the binding of dioxin-like  
241 HAHs, e.g. dioxin-like PCBs (dl-PCBs), to the Aryl hydrocarbon receptor (AhR) via  
242 activation of a reporter gene. The assay was performed by BioDetection Systems b.v.  
243 (Amsterdam, The Netherlands).

244

245 Bioassay-derived TEQ values, or bioequivalent values (BEQs), were compared with TEQ  
246 values calculated from the chemical analytical data. This comparison revealed if there was a  
247 significant higher activity than predicted from the chemical analysis. Such a combined  
248 approach has been repeatedly used in studies on chemical contamination of the Arctic region  
249 (Letcher et al., 2010), but it has not been used in studies with Antarctic fish so far.

250

## 251 2.5 Calculations and statistics

252

253 All analyses include the lipid content of the respective tissue. POP concentrations were  
254 normalized against both lipid weight (lw, ng g<sup>-1</sup>) and fresh weight (fw, ng g<sup>-1</sup>) of the tissue  
255 and provided as mean values ± standard error of the mean (sem). Toxicant concentrations  
256 were compared among icefish species and tissues using ANOVA with Tukey post-hoc test.



257 Influence of sampling site, fish weight and length, maturity stage and tissue lipid content was  
258 tested with ANOVA. Data were considered to be statistically significant at  $p < 0.05$ . Normal  
259 distribution of data was tested with Kolmogorov-Smirnov and equality of variances with  
260 Bartlett's test. All statistical tests were performed with STATISTICA 12, StatSoft, Inc., and  
261 GraphPad Prism 5, GraphPad Software, Inc.

262

263

### 264 3. Results

265

#### 266 3.1 Interspecies comparison of contaminant patterns and levels

267

268 In this study, we measured lipid content, PCB, DDT, HCB,  $\gamma$ -HCH, and PBDE concentrations  
269 in both muscle and ovary tissue of female, mature *C. gunnari* and *C. aceratus* from spatially  
270 closely located sampling sites around Elephant Island and the South Shetland Islands. We  
271 express the compound concentrations on lipid weight and the fresh weight basis.

272 Sampling site, maturity stage and tissue lipid content of the fish showed no correlation to the  
273 accumulation of any of the compounds analysed in this study.

274 All samples contained detectable levels of the target compounds, which are presented in  
275 Table 1. Analytes concentrations were about 15 - 110 times higher when calculated per lipid  
276 weight than based on tissue fresh weight.

277 Overall, the PCBs were the predominant group among all compounds analysed in our study  
278 (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  $20.0 \pm 4.3$ , *C. gunnari* ovaries  $47.8 \pm$   
279  $10.2$ , *C. aceratus* muscle  $21.9 \pm 4.9$ , *C. aceratus* ovaries  $31.9 \pm 7.3$ ), followed by the DDTs  
280 (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  $7.0 \pm 1.0$ , *C. gunnari* ovaries  $6.9 \pm 1.1$ , *C.*  
281 *aceratus* muscle  $19.4 \pm 8.1$ , *C. aceratus* ovaries  $17.8 \pm 3.9$ ), HCB (mean values  $\pm$  sem ( $\text{ng g}^{-1}$   
282 lw): *C. gunnari* muscle  $3.8 \pm 0.5$ , *C. gunnari* ovaries  $2.8 \pm 0.4$ , *C. aceratus* muscle  $6.2 \pm 0.9$ ,  
283 *C. aceratus* ovaries  $7.3 \pm 2.0$ ), the PBDEs (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  
284  $4.1 \pm 0.5$ , *C. gunnari* ovaries  $0.7 \pm 0.1$ , *C. aceratus* muscle  $5.1 \pm 0.8$ , *C. aceratus* ovaries  $10.0$   
285  $\pm 1.9$ ) and  $\gamma$ -HCH (mean values  $\pm$  sem ( $\text{ng g}^{-1}$  lw): *C. gunnari* muscle  $0.6 \pm 0.2$ , *C. gunnari*  
286 ovaries  $1.3 \pm 0.3$ , *C. aceratus* muscle  $0.6 \pm 0.2$ , *C. aceratus* ovaries  $1.2 \pm 0.5$ ) (Figure 1).

287 Within the PCBs, only PCB 28 and 101 were significantly different between the ovaries of *C.*  
288 *gunnari* and *C. aceratus* (Table 1). When we calculated the percentage contribution of the  
289 individual PCB congeners to  $\sum$ PCBs (lw) of both muscle and ovary tissue, there was no

290 difference in PCB congener composition between the two icefish species (two-way  
291 ANOVA).  
292 Among the PCBs, PCB 153 contributed by 28% in muscle and by 26% in ovaries to all PCBs  
293 in both species. The second-most abundant PCBs were PCB 138 (~26%) and 101 (~21%)  
294 (Figure 2).

295

296 In the ovaries, levels of *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT (per lw) were significantly  
297 higher in *C. aceratus* compared to those of *C. gunnari*. Also *p,p'*-DDT concentrations in the  
298 muscle of *C. aceratus* were higher than those in muscle of *C. gunnari* (per lw).  $\Sigma$ DDT (per  
299 lw) was significantly higher in muscle and ovaries of *C. aceratus* than in *C. gunnari* (Figure  
300 1). Among the DDTs, *p,p'*-DDE showed the highest concentration of up to 57% in muscle of  
301 *C. aceratus*. *p,p'*-DDE tended to be higher in muscle tissue, while *p,p'*-DDT was slightly  
302 higher in ovary tissue in both species. *o,p'*-DDT was slightly higher in *C. gunnari* with  
303 ~22%, while it was only ~16% in *C. aceratus*. Detailed information on the contribution of all  
304 individual compounds measured in this study is given in Table A.2 in the appendix.

305  $\gamma$ -HCH was only different between the muscle tissues of the two icefish (on fw basis).

306 HCB concentrations per lipid weight in both muscle and ovaries were higher in *C. aceratus*  
307 than in *C. gunnari* (Figure 1). Except HCB, none of the analysed compounds exhibited a  
308 correlation with fish weight or length. HCB showed a significant linear dependence of fish  
309 weight and length in *C. aceratus*, except in the ovaries ( $R^2=0.723$ ).

310

311 In both species, the PBDEs were dominated by BDE 47 (~59% in muscle, ~54% in ovaries),  
312 followed by ~20% BDE 99. On both the lipid and the fresh weight basis, BDE 183 and 197  
313 were significantly different between the two tissues types and fish species. Also BDE 99  
314 showed different concentrations between the ovaries of *C. aceratus* and *C. gunnari* on the  
315 lipid basis (Table 1, Table A.2, Figure 2).

316

### 317 3.2 Comparison of contaminant patterns and levels in different tissue

318

319 Lipid content was significantly higher in the ovaries of *C. gunnari* than in the ovaries of *C.*  
320 *aceratus*. *C. gunnari* exhibited significantly higher lipid content in ovaries than in their  
321 muscle tissue, while this was not the case for *C. aceratus* (Table 1).

322 A comparison between the compound concentrations in muscle and ovary tissue for each  
323 icefish species revealed higher HCB concentrations in ovary than in muscle tissue of *C.*

324 *gunnari*. Concentrations of  $\gamma$ -HCH and DDTs in muscle and ovary tissue (per fresh weight)  
325 were significantly different in both species (Table 1).

326 In *C. aceratus*, all PCB concentrations and most of the PBDEs were different from each other  
327 in both muscle and ovary tissue. In case of dl-PCB congeners, the  $\Sigma$ dl-PCBs were higher in  
328 muscle of *C. gunnari* than in muscle *C. aceratus* on both lipid and fresh weight basis (Table  
329 A.1).

330 In *C. gunnari*, all PBDEs apart from congener 183 did differ between muscle and ovary  
331 tissue. In contrast, only PCB 28 and 180 were different between the tissues of *C. gunnari*, all  
332 other PCB congeners showed no significant tissue differences (Table 1, Figure 1).

333

### 334 3.3 Toxic equivalents (TEQs)

335

336 The toxic equivalent levels of dioxin-like PCBs ( $WHO_{PCB-TEQ} g^{-1} fw$ ) were higher in the  
337 muscle of *C. gunnari* ( $0.5 \pm 0.1$ ) than in *C. aceratus* ( $0.2 \pm 0.1$ ), while *C. aceratus* exhibited  
338 higher TEQs ( $0.5 \pm 0.9$ ) in the ovaries than *C. gunnari* ( $0.1 \pm 0.0$ ) (Table 1).

339

### 340 3.4 Bioanalytical equivalents (BEQs)

341

342 The bioanalytical equivalents (BEQs) determined by DR-CALUX in the muscle of both  
343 species were in a similar range (*C. aceratus*:  $0.15 \pm 0.07$ , *C. gunnari*:  $0.2 \pm 0.14$  BEQ  $g^{-1} fw$ )  
344 to the  $WHO_{PCB-TEQs} g^{-1} fw$ , which were calculated using the concentrations of dioxin-like  
345 PCBs. In *C. gunnari*, however, the BEQs measured in the ovaries were about 70-fold higher  
346 (BEQ:  $5.14 \pm 3.6$ , TEQ:  $0.07 \pm 0.04$ ) than the calculated  $WHO_{PCB-TEQ} g^{-1} fw$  (Table 1).

347

348

## 349 4. Discussion

350

### 351 4.1 Species-specific contaminant patterns and levels

#### 352 4.1.1. Species-specific patterns

353

354 Literature data on present POP concentrations in tissues of Antarctic fish are scarce, and  
355 particularly those on contaminant levels in white-blooded icefish.

356 Earlier studies of the 80's and 90's measured PCB concentrations in the icefish *C. gunnari*  
357 and *C. aceratus* around the Antarctic Peninsula (Corsolini et al. 2002a, 2002b, 2009), which

358 were two to four times lower ( $0.7 \text{ ng g}^{-1}$  fw in muscle) than the ones of the present study.  
359 HCB levels of our icefish were within a similar range to those measured previously. It is  
360 known for sub-Antarctic fish that PCB levels can follow a seasonal trend, which is related to  
361 the release of pollutants from melting snow and ice during summer and the atmospheric  
362 transport of loads of pollutants which precipitate in Antarctic regions and are released during  
363 warming (Jaffal et al., 2011). Since sampling of our icefish took place in Austral autumn,  
364 after the seasonal ice melting in Antarctica, a seasonal release of POPs trapped in sea ice  
365 could thereby be one contributor to the comparably high levels of PCBs in our icefish  
366 samples. In fact, the PCB concentrations (per fw) of our icefish were within a similar range to  
367 those of salmon from the Baltic Sea (Isosaari et al., 2006).

368 In the early 00's, Borghesi et al. (2008; 2009) sampled *C. hamatus* and *C. gunnari* in the  
369 Ross Sea at  $74^\circ$  South. They found  $\Sigma$ PCB concentrations in *C. hamatus* muscle of  $0.35 \text{ ng g}^{-1}$   
370 fw,  $\Sigma$ non-ortho PCBs of  $5 \text{ ng g}^{-1}$  fw and  $\Sigma$ PBDEs  $0.16 \text{ ng g}^{-1}$  fw. In muscle of *C. gunnari*,  
371 they report  $\Sigma$ PBDEs of  $0.44 \text{ ng g}^{-1}$  fw. Those values are about five times higher than the  
372  $\Sigma$ PBDEs in muscle of *C. gunnari* measured in our study. Since they used fish of a similar  
373 size class, this difference is likely related to the measurement of different PBDE congeners in  
374 the studies.

375 Another recent study measured contaminant concentrations (in  $\text{ng g}^{-1}$  lw) in muscle, liver and  
376 gonads of three red-blooded Antarctic species, *Notothenia coriiceps*, *N. rossii* and  
377 *Trematomus newnesi*, from Potter Cove, Antarctic Peninsula (Lana et al., 2014). They  
378 reported similar  $\Sigma$ DDT values in muscle, but also species differences in the ovaries of their  
379 fish: while *N. coriiceps* and *T. newnesi* had similar values to *C. aceratus* measured in our  
380 study, *N. rossii* had much higher values than our fish. They also measured  $\gamma$ -HCH values  
381 about six-times higher than in our fish. *N. rossii* displays a rather benthic lifestyle, but also  
382 feeds on pelagic species, and thus has a prey spectrum similar to *C. gunnari*. Nevertheless,  
383 the DDTs and  $\gamma$ -HCH were much higher in *N. rossii* than in *C. gunnari*. In contrast,  $\Sigma$ PCB  
384 and  $\Sigma$ BDEs were highly variable among the species and tissues measured by Lana et al.  
385 (2014), but were generally within the same order of magnitude compared to our data. Thus,  
386 such strong differences in DDT and  $\gamma$ -HCH accumulation patterns between Antarctic fish  
387 species could also be related to a selective metabolism for individual contaminant classes  
388 between the species (Storelli et al., 2009), and not only to their ecological traits.

389

390 Amongst the PBDEs, BDE 47 showed the highest concentration (60% of all congeners) in  
391 our icefish amongst all PBDE congeners, followed by BDE 100 and BDE 99. This is in line

392 with the general picture of those congeners being the dominating PBDEs in fish around the  
393 globe, i.e. BDE 47 being recognized as the most important PBDE congener in marine biota  
394 (Zennegg et al., 2003; Isosaari et al., 2006; Kuiper et al., 2006).

395 In comparison to fish from non-Antarctic regions, PBDE concentrations on the fresh weight  
396 basis were particularly low in icefish. For example, PBDE concentrations range from about  
397 1.0 to 8 ng g<sup>-1</sup> fresh weight, or up to 64 ng g<sup>-1</sup> lipid weight in various fish species from the  
398 Baltic Sea (Isosaari et al., 2006), which are at least ten times higher than in the icefish.

399 Although the production of the former widely used brominated flame retardants penta- and  
400 octabromodiphenyl ether (PentaBDE and OctaBDE) were banned by the European Union in  
401 2004 and several states of the USA, toxic and persistent lower brominated PBDEs are still  
402 produced in other areas of the world and redistributed globally, also to the Antarctic (Cox and  
403 Efthymiou, 2003; Renner, 2004; Vives et al., 2004; Kuiper et al., 2006).

404 HCB concentrations (per fw) were also up to 30 times lower, and DDT concentrations (per  
405 fw) several hundreds of times lower in our icefish than in fish from the Northern hemisphere  
406 (Sharma et al., 2009). Despite a worldwide stop of the production of DDTs during the 70's, it  
407 has been reintroduced in the 2000s as malaria control by the WHO, and about 6000 tons of  
408 DDTs are still produced per year (UNEP, 2008). Due to its high persistence, bioaccumulation  
409 potential and cold condensation processes, DDTs and its metabolites are nowadays found in  
410 biota all over the world, and particularly in polar regions (Mirmigkou and de Boer, 2015).

411

412 The TEQs we calculated for *C. aceratus* and *C. gunnari* were in a similar range than TEQs  
413 reported for muscle of the icefish *C. hamatus* (TEQ 0.01-0.1 pg g<sup>-1</sup> wet weight) or red-  
414 blooded Antarctic fish (TEQ ~0.1 pg g<sup>-1</sup> wet weight) (Focardi et al.; Corsolini et al., 2002a;  
415 Borghesi et al., 2008). Generally, the values for Antarctic fish were lower than those for other  
416 organisms living in less remote parts of the world, e.g. WHO<sub>PCB</sub>-TEQ (pg g<sup>-1</sup> fw) of 3 to 15  
417 in muscle of salmon or Baltic herring. Yet, burbot from Bothnian bay (Baltic Sea) exhibit  
418 muscle TEQs, which are in a similar range as in Antarctic fish (Isosaari et al., 2006).

419 In *C. aceratus*, we found that the BEQs were comparable to the TEQs calculated on the basis  
420 on the WHO<sub>PCB</sub>-TEFs for salmonid fish species (Van den Berg et al., 1998). In comparison to  
421 fish from the northern hemisphere (Husain et al., 2014), Antarctic fish had about twenty  
422 times lower BEQ values in their muscle. In contrast, the BEQs in the ovaries of *C. gunnari*  
423 were much higher than the WHO<sub>PCB</sub>-TEQs calculated for their ovaries. The toxicity effects  
424 might thus be actually much higher than expected by the single usage of the calculated  
425 WHO<sub>PCB</sub>-TEQ. In fact, the BEQs in the ovaries of *C. gunnari* were within the same range as

426 BEQs of fish from temperate latitudes. Also Corsolini et al. (Corsolini et al., 2002a) stated  
427 that the TEQ values they measured in Antarctic fish were already half as high as those values  
428 which are considered to elicit toxicological effects, such as reproductive and immunological  
429 disorders, in marine mammals or birds (Kannan et al., 2000).

430 Rising PCB concentrations as observed by us in tissues of Antarctic fish will thus  
431 increasingly have the potential to exert their toxic effects on those fish.

432

#### 433 4.1.2. Temporal trends in contaminant levels

434

435 Long-term observations on contaminant levels in Antarctic biota are scarce. Yet, Weber and  
436 Goerke (2003; Goerke et al., 2004) measured contaminant levels in the liver of *C. aceratus*  
437 and *C. gunnari* in the same sampling area as in the present study. From 1987 to 1996, the  
438 authors measured an increase of PCB 153 and PCB 180 levels in *C. aceratus*, but not in *C.*  
439 *gunnari*. In the present study, we found about three times higher concentrations of those PCB  
440 congeners in the tissues of our two icefish species than in the previous study from 1996  
441 (Weber and Goerke, 2003). In contrast, the HCB concentrations show a declining trend from  
442 the 1987 study to our current survey. A similar trend of stable or declining HCB levels by up  
443 to 2.5% per year has also been observed in Arctic biota, such as birds, fish or marine  
444 mammals, since the late 80's (Barber et al., 2005; Rigét et al., 2010).

445

446 In liver of *C. aceratus*, concentrations of *p,p'*-DDE had already increased from 1987 to 1996,  
447 and the values we measured in muscle and ovaries of *C. aceratus* were almost twice as high  
448 as in 1996 (Weber and Goerke, 2003). In contrast, *p,p'*-DDE concentrations in *C. gunnari*  
449 remained at similar levels from 1987 over 1996 (study by Weber and Goerke (2003)) to the  
450 present study.

451 Nevertheless, overall DDT concentrations were increasing from 1987 to 1996 in both icefish  
452 species, and our values are also slightly higher than those in 1996, suggesting an increasing  
453 trend of DDTs in icefish around the Antarctic Peninsula. Despite a general global reduction  
454 of DDT as an insecticide, DDT is still produced at high volumes (see above, (UNEP, 2008)).  
455 Furthermore, climate warming on the one hand leads to an increased volatility and worldwide  
456 distribution of DDTs, and on the other hand local sources such as melting glaciers may  
457 additionally contribute to increasing DDT concentrations in tissues of Antarctic fish around  
458 the Antarctic Peninsula (van den Brink et al., 2009; van den Brink et al., 2011).

459

## 460 4.2 Correlation of contaminant concentrations with ecological traits

461

### 462 4.2.1 Tissue-specific patterns

463

464 Since all POPs analyzed in this study are highly lipophilic substances, tissue differences in  
465 the contaminant levels in *C. aceratus* and *C. gunnari* on fresh weight basis at first instance  
466 should correlate to tissue lipid concentrations (Corsolini et al., 2002a; Weber and Goerke,  
467 2003; Chiuchiolo et al., 2004). Indeed, *C. gunnari*, which possesses a clearly higher lipid  
468 content in the ovaries than *C. aceratus*, accordingly showed significantly higher PCB  
469 concentrations in its ovaries based on fresh weight compared to *C. gunnari*. Also HCB was  
470 two times more concentrated in the fat-rich ovaries of *C. aceratus* than in *C. gunnari*, but  
471 only on the lipid basis. Accordingly, the BEQs were higher in the ovaries of *C. aceratus* and  
472 *C. gunnari* than in their muscle tissue. Also Lana et al. (2014), investigating POP  
473 accumulation patterns in notothenioid species, reported that the highest levels were found in  
474 the gonads of the fish.

475

### 476 4.2.2 Ecological-related patterns

477

478 In addition to body lipid contents, our results point to an influence of habitat and trophic level  
479 on POP levels in white-blooded icefish. The benthic-living *C. aceratus* had two-times higher  
480 concentrations of almost all DDT congeners and HCB than the benthopelagic *C. gunnari*.  
481 Also the previous study by Weber and Goerke (2003) report higher (lipid-based) contaminant  
482 burdens in *C. aceratus* than in *C. gunnari* and highlight the higher tendency of *C. aceratus* to  
483 accumulate DDTs in its tissues than *C. gunnari* over the time.

484 Since POPs accumulate in sediments, benthic fish species are generally thought have a higher  
485 exposure and uptake of lipophilic contaminants (Goerke et al., 2004; Borghesi et al., 2008).

486 In addition to uptake from water or sediment, also the feeding habit thus plays a role, with  
487 species at higher trophic levels tending to show higher contaminant accumulation due to  
488 biomagnification. A recent study by Wolschke et al. (2015) also highlights the  
489 biomagnification of POPs from lower to higher trophic levels in the Antarctic food chain,  
490 which can be attributed to the diets of the animals.

491 From these observations, the benthic, piscivorous *C. aceratus* was expected to have higher  
492 contaminant burdens than the benthopelagic, planktivorous *C. gunnari*. However, only HCB

493 and DDTs, but none of the other congener classes, were higher in the predominantly benthic  
494 *C. aceratus* than in the benthopelagic *C. gunnari* on the lipid weight basis.

495

496

## 497 **Conclusion**

498

499

500 Overall, PCB and DDT concentrations tend to rather increase than decrease in tissues of the  
501 two white-blooded icefish species *C. aceratus* and *C. gunnari* around Elephant Island and the  
502 South Shetland Islands, when compared to earlier studies. Our results thereby support the  
503 global transportation of POPs to the Southern Ocean and their bioaccumulation in the local  
504 marine fish, and point to a trend of increasing concentrations of POPs in Antarctic icefish.  
505 Our data also suggest that worldwide climate change effects may contribute to an increased  
506 volatilization and release of POPs trapped in glaciers, sea- or pack-ice, thereby leading to an  
507 ongoing contamination of the Southern Ocean and its biota.

508 Furthermore, we found differences in POP accumulation patterns between the two icefish  
509 species, which were weakly correlated to their trophic position. The piscivorous *C. aceratus*  
510 showed a higher potential to accumulate contaminants in its tissue than the planktivorous *C.*  
511 *gunnari*. This species difference highlights the influence of intake of POPs via the specific  
512 prey of individual fish species. However, the expected link between the contaminant burdens  
513 of *C. aceratus* and *C. gunnari* and their ecological traits could not be fully supported.

514 Additional factors, such species differences in toxicant metabolism rates and selective  
515 metabolism for single contaminant classes, may also play an important role in defining  
516 chemical bioaccumulation patterns in Antarctic fish species in the long term. In the end,  
517 Antarctic fish are a central link between the benthic community and top level predators,  
518 concomitantly POPs bioaccumulated in their tissues are likely to contribute to a progressive  
519 biomagnification of POPs along Antarctic food webs.

520

521

## 522 **Acknowledgements**

523 This work was funded by the Swiss National Science Foundation (SNSF 31003A\_149964/1)  
524 and the Freiwillige Akademische Gesellschaft Basel. We thank Donatella Perrone and  
525 Melanie Senn (Empa) for their helpful assistance in the laboratory.

526



527 **Tables & Figures**

528

529 **Tables**

530

531 **Table 1:** Lipid content (%) and levels of organic contaminants (ng g<sup>-1</sup> lipid weight; ng g<sup>-1</sup> fresh weight) (mean ± sem) in tissues of two Antarctic  
 532 icefish species.

533

	<i>C. aceratus</i> (n=10)				<i>C. gunnari</i> (n=11)					
Length (cm)	47-66				33-50					
Weight (g)	636-3620				252-888					
	lipid weight (ng g <sup>-1</sup> lw)		fresh weight (ng g <sup>-1</sup> fw)		lipid weight (ng g <sup>-1</sup> lw)		fresh weight (ng g <sup>-1</sup> fw)		Blank	
Tissue	muscle	ovaries	muscle	ovaries	muscle	ovaries	muscle	ovaries		
	mean	sem	mean	sem	mean	sem	mean	sem		
<b>TEQs</b>			<b>0.16</b>	<b>0.1</b>	<b>0.53</b>	<b>0.94</b>	<b>0.47</b>	<b>0.14*</b>	<b>0.07</b>	<b>0.04*</b>
<b>BEQs</b>			<b>0.15</b>	<b>0.07</b>	<b>0.57</b>	<b>0.75</b>	<b>0.20</b>	<b>0.14*</b>	<b>5.14</b>	<b>3.60*</b>

HCB	6.22	0.85 <sup>#</sup>	7.37	1.98 <sup>#</sup>	0.09	0.02	0.16	0.04	3.82	0.55 <sup>#</sup>	2.84	0.37 <sup>#</sup>	0.07	0.01 <sup>*</sup>	0.18	0.04 <sup>*</sup>	0.06
γ-HCH	0.58	0.21 <sup>*</sup>	1.18	0.49 <sup>*</sup>	0.01	<0.01 <sup>#,*</sup>	0.03	0.01 <sup>*</sup>	0.59	0.20	1.25	0.31	0.01	<0.01 <sup>#,*</sup>	0.07	0.02 <sup>*</sup>	0.07
<i>p,p'</i> -DDE	13.11	5.99	8.76	2.46 <sup>#</sup>	0.14	0.04 <sup>#,*</sup>	0.19	0.03 <sup>*</sup>	3.19	0.58	2.36	0.38 <sup>#</sup>	0.06	0.01 <sup>#,*</sup>	0.16	0.04 <sup>*</sup>	0.52
<i>o,p'</i> -DDT	3.20	0.98	2.83	0.65 <sup>#</sup>	0.04	0.01 <sup>*</sup>	0.07	0.01 <sup>*</sup>	1.65	0.36	1.64	0.38 <sup>#</sup>	0.03	<0.01 <sup>*</sup>	0.09	0.02 <sup>*</sup>	0.07
<i>p,p'</i> -DDT	5.31	1.61 <sup>#</sup>	6.21	1.10 <sup>#</sup>	0.06	0.02 <sup>*</sup>	0.15	0.02 <sup>*</sup>	2.19	0.42 <sup>#</sup>	2.98	0.52 <sup>#</sup>	0.04	0.01 <sup>*</sup>	0.19	0.04 <sup>*</sup>	0.32
<b>Σ DDTs</b>	<b>19.45</b>	<b>8.14<sup>#</sup></b>	<b>17.80</b>	<b>3.91<sup>#</sup></b>	<b>0.22</b>	<b>0.07</b>	<b>0.40</b>	<b>0.05</b>	<b>7.04</b>	<b>1.00<sup>#</sup></b>	<b>6.98</b>	<b>1.14<sup>#</sup></b>	<b>0.12</b>	<b>0.02<sup>*</sup></b>	<b>0.44</b>	<b>0.10<sup>*</sup></b>	
28	0.95	0.06 <sup>*</sup>	1.50	0.26 <sup>*</sup>	0.01	<0.01 <sup>*</sup>	0.04	0.01 <sup>#,*</sup>	1.23	0.11 <sup>*</sup>	2.84	0.53 <sup>*</sup>	0.04	0.01 <sup>*</sup>	0.18	0.05 <sup>#,*</sup>	0.12
52	2.15	0.79 <sup>*</sup>	3.29	0.98 <sup>*</sup>	0.02	0.01 <sup>*</sup>	0.09	0.03 <sup>*</sup>	1.56	0.38	7.72	2.31	0.36	0.34	0.37	0.09	0.25
101	5.12	1.48 <sup>*</sup>	7.10	1.69 <sup>*</sup>	0.06	0.01 <sup>*</sup>	0.19	0.05 <sup>#,*</sup>	3.99	0.97	10.74	2.72	0.68	0.63	0.60	0.12 <sup>#</sup>	0.26
138	5.74	1.21 <sup>*</sup>	8.20	1.83 <sup>*</sup>	0.07	0.01 <sup>*</sup>	0.22	0.05 <sup>*</sup>	5.47	1.23	11.06	2.69	0.60	0.52	0.68	0.18	0.40
153	6.00	1.26 <sup>*</sup>	8.72	2.10 <sup>*</sup>	0.07	0.01 <sup>*</sup>	0.23	0.06 <sup>*</sup>	5.94	1.40	11.96	2.52	0.41	0.33	0.76	0.20	0.49
180	1.94	0.31 <sup>*</sup>	3.06	0.67 <sup>*</sup>	0.02	<0.01 <sup>*</sup>	0.08	0.02 <sup>*</sup>	1.86	0.35 <sup>*</sup>	3.51	0.50 <sup>*</sup>	0.07	0.04	0.25	0.08	0.17
<b>Σ PCBs</b>	<b>21.91</b>	<b>4.98<sup>*</sup></b>	<b>31.87</b>	<b>7.26<sup>*</sup></b>	<b>0.26</b>	<b>0.03<sup>*</sup></b>	<b>0.85</b>	<b>0.21<sup>#,*</sup></b>	<b>20.04</b>	<b>4.27</b>	<b>47.82</b>	<b>10.24</b>	<b>2.17</b>	<b>1.86</b>	<b>2.85</b>	<b>0.63<sup>#</sup></b>	
28	0.21	0.09	0.83	0.37	<0.01	<0.01 <sup>*</sup>	0.02	0.01 <sup>*</sup>	0.22	0.08 <sup>*</sup>	0.87	0.33 <sup>*</sup>	<0.01	<0.01 <sup>*</sup>	0.05	0.02 <sup>*</sup>	<0.01

47	2.90	0.50*	5.32	1.10*	0.04	<0.01*	0.15	0.03*	2.46	0.39*	10.14	1.41*	0.04	0.01*	0.70	0.26*	0.33
100	0.59	0.12*	1.27	0.27*	0.01	<0.01*	0.04	0.01*	0.45	0.10*	2.17	0.42*	0.01	<0.01*	0.15	0.05*	0.11
99	0.99	0.47*	1.97	0.49 <sup>#,*</sup>	0.01	0.02*	0.06	0.02*	0.86	0.12*	5.28	1.01 <sup>#,*</sup>	0.02	<0.01*	0.39	0.19*	0.05
153	0.11	0.02	0.21	0.07	<0.01	<0.01*	0.01	<0.01*	0.07	0.01*	0.53	0.09*	<0.01	<0.01*	0.04	0.02*	0.01.
183	0.08	0.01 <sup>#</sup>	0.16	0.07	<0.01	<0.01 <sup>#,*</sup>	<0.01	<0.01 <sup>#,*</sup>	0.04	0.01 <sup>#</sup>	0.53	0.09	<0.01	<0.01 <sup>#,*</sup>	0.04	0.02 <sup>#,*</sup>	0.01
197	0.13	0.03 <sup>#,*</sup>	0.27	0.08 <sup>#,*</sup>	<0.01	<0.01*	0.01	<0.01 <sup>#,*</sup>	0.07	0.01 <sup>#,*</sup>	0.33	0.10 <sup>#,*</sup>	<0.01	<0.01*	0.02	0.01 <sup>#,*</sup>	0.01
<b>Σ PBDEs</b>	<b>5.01</b>	<b>0.82*</b>	<b>10.03</b>	<b>1.86*</b>	<b>0.07</b>	<b>0.01*</b>	<b>0.29</b>	<b>0.06*</b>	<b>4.08</b>	<b>0.54*</b>	<b>0.72</b>	<b>0.14*</b>	<b>0.08</b>	<b>0.01*</b>	<b>0.05</b>	<b>0.02*</b>	
%lipid					1.45	0.18	2.64	0.23 <sup>#</sup>					2.13	0.35*	6.93	1.32 <sup>#,*</sup>	

534 In **bold**: sum (Σ) of all DDT, PCB & PBDE congeners. Lw: lipid weight, fw: fresh weight. TEQ: toxic equivalents, pg WHO-TEQ<sup>-1</sup>g fw,  
535 calculated by using the toxic equivalency factors recommended by Van den Berg (1998). BEQ: bioequivalent values, BEQ g<sup>-1</sup> fw. The # denotes a  
536 significant difference between *C. gunnari* and *C. aceratus* in the given tissue at  $p \leq 0.05$ . The \* denotes a significant difference between tissues for  
537 each species at  $p \leq 0.05$ .

538 **Figure Captions**

539

540 **Figure 1** Mean ( $\pm$  sem) concentration of  $\Sigma$  PCBs,  $\Sigma$  BDEs, HCBs,  $\gamma$ -HCHs and  $\Sigma$  DDTs (ng  
541  $\text{g}^{-1}$  lipid weight), in muscle and ovaries of the two icefish species, *C. gunnari* ( $n=11$ ) and *C.*  
542 *aceratus* ( $n=10$ ). The # denotes a significant difference between *C. gunnari* and *C. aceratus* in  
543 the given tissue at  $p \leq 0.05$ . The \* denotes a significant difference between tissues for each  
544 species at  $p \leq 0.05$ .

545

546 **Figure 2:** Congener composition (percentage) of PCBs, DDTs and BDEs in muscle (m) and  
547 ovaries (ov) of *C. gunnari* (Cg) and *C. aceratus* (Ca).

548

549

551 **References**

- 552 Barber, J.L., Sweetman, A.J., Van Wijk, D., Jones, K.C., 2005. Hexachlorobenzene in the  
 553 global environment: emissions, levels, distribution, trends and processes. *Sci. Total. Environ.*  
 554 349, 1-44.
- 555 Bargagli, R., 2008. Environmental contamination in Antarctic ecosystems. *Sci. Total.*  
 556 *Environ.* 400, 212-226.
- 557 Bogdal, C., Nriagu, D., Luthy, M.P., Schenker, S., Scheringer, M., Hungerbühler, K., 2010.  
 558 Release of legacy pollutants from melting glaciers: model evidence and conceptual  
 559 understanding. *Environ. Sci. Technol.* 44, 4063-4069.
- 560 Borghesi, N., Corsolini, S., Focardi, S., 2008. Levels of polybrominated diphenyl ethers  
 561 (PBDEs) and organochlorine pollutants in two species of Antarctic fish (*Chionodraco*  
 562 *hamatus* and *Trematomus bernacchii*). *Chemosphere* 73, 155-160.
- 563 Borghesi, N., Corsolini, S., Leonards, P., Brandsma, S., de Boer, J., Focardi, S., 2009.  
 564 Polybrominated diphenyl ether contamination levels in fish from the Antarctic and the  
 565 Mediterranean Sea. *Chemosphere* 77, 693-698.
- 566 Cabrerizo, A., Dachs, C., Barcel, D., Jones, K.C., 2013. Climatic and biogeochemical  
 567 controls on the remobilization and reservoirs of persistent organic pollutants in Antarctica.  
 568 *Environ. Sci. Technol.* 47, 4299-4306.
- 569 Chiuchiolo, A.L., Dickhut, R.M., Cochran, M.A., Ducklow, H.W., 2004. Persistent organic  
 570 pollutants at the base of the Antarctic marine food web. *Environ. Sci. Technol.* 38, 3551-  
 571 3557.
- 572 Corsolini, S., Borghesi, N., Schiamone, A., Focardi, S., 2007. Polybrominated diphenyl  
 573 ethers, polychlorinated dibenzo-dioxins,-furans, and-biphenyls in three species of Antarctic  
 574 penguins. *Environ. Sci. Pollut. R.* 14, 421-429.
- 575 Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., Giesy, J.P., 2002a.  
 576 Polychloronaphthalenes and other dioxin-like compounds in Arctic and Antarctic marine food  
 577 webs. *Environ. Sci. Technol.* 36, 3490-3496.
- 578 Corsolini, S., Ademollo, N., Mariottini, M., Fossi, S., Guerranti, C., Perra, G., Duhamel, G.,  
 579 Focardi, S., 2002b. Polychlorinated biphenyls, polychlorinated-dibenzodioxins and –  
 580 dibenzofurans in mackerel icefish and marbled rockcod from the Kerguelen Islands  
 581 (Antarctica). *Food and Feed I*, 57, 161 – 164.
- 582 Cox, P., Efthymiou, P., 2003. Directive 2003/11/EC of the European parliament and of the  
 583 council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC  
 584 relating to restrictions on the marketing and use of certain dangerous substances and  
 585 preparations (pentabromodiphenyl ether, octabromodiphenyl ether). *Off. J. Eur. Union* 42, 45-  
 586 46.
- 587 Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for  
 588 upwelling of corrosive "acidified" water onto the continental shelf. *Science* 320, 1490-1492.
- 589 Focardi, S., Fossi, M., Lari, L., Casini, S., Leonzio, C., Meidel, S., Nigro, M., 1995. Induction  
 590 of MFO activity in the antarctic fish *Pagothenia bernacchii*: Preliminary results. *Mar.*  
 591 *Environ. Res.* 39, 97-100.
- 592 Goerke, H., Weber, K., Bornemann, H., Ramdohr, S., Plötz, J., 2004. Increasing levels and  
 593 biomagnification of persistent organic pollutants (POPs) in Antarctic biota. *Mar. Pollut. Bull.*  
 594 48, 295-302.
- 595 Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G., 2013.  
 596 Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica.  
 597 *Mar. Pollut. Bull.* 77, 82-89.

598 Hale, R.C., Kim, S.L., Harvey, E., La Guardia, M.J., Mainor, T.M., Bush, E.O., Jacobs, E.M.,  
599 2008. Antarctic research bases: local sources of polybrominated diphenyl ether (PBDE) flame  
600 retardants. *Environ. Sci. Technol.* 42, 1452-1457.

601 Hartmann, R., 2013. Extraction of PCDD/Fs and PCBs in fish using SpeedExtractor E-914.  
602 *Proc. Environ. Sci.* 18, 875-881.

603 Husain, A., Gevao, B., Dashti, B., Brouwer, A., Behnisch, P.A., Al-Wadi, M., Al-Foudari,  
604 M., 2014. Screening for PCDD/Fs and dl-PCBs in local and imported food and feed products  
605 available across the State of Kuwait and assessment of dietary intake. *Ecotox. Environ. Safe.*  
606 100, 27-31.

607 Isoaari, P., Hallikainen, A., Kiviranta, H., Vuorinen, P.J., Parmanne, R., Koistinen, J.,  
608 Vartiainen, T., 2006. Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls,  
609 naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea  
610 and lakes in Finland. *Environ. Pollut.* 141, 213-225.

611 Jaffal, A., Paris-Palacios, S., Jolly, S., Thailly, A., Delahaut, L., Beall, E., Roche, H.,  
612 Biagianti-Risbourg, S., Betoulle, S., 2011. Cadmium and copper contents in a freshwater fish  
613 species (brook trout, *Salvelinus fontinalis*) from the subantarctic Kerguelen Islands. *Polar*  
614 *Biol.* 34, 397-409.

615 Kannan, K., Blankenship, A., Jones, P., Giesy, J., 2000. Toxicity reference values for the  
616 toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum. Ecol. Risk Assess.* 6,  
617 181-201.

618 Kohler, M., Zennegg, M., Bogdal, C., Gerecke, A.C., Schmid, P., V. Heeb, N., Sturm, M.,  
619 Vonmont, H., E. Kohler, H.-P., Giger, W., 2008. Temporal trends, congener patterns, and  
620 sources of octa-, nona-, and decabromodiphenyl ethers (PBDE) and  
621 hexabromocyclododecanes (HBCD) in Swiss lake sediments. *Environ. Sci. Technol.* 42,  
622 6378-6384.

623 Kuiper, R.V., Murk, A., Leonards, P.E., Grinwis, G., Van den Berg, M., Vos, J.G., 2006. In  
624 vivo and in vitro Ah-receptor activation by commercial and fractionated  
625 pentabromodiphenylether using zebrafish (*Danio rerio*) and the DR-CALUX assay. *Aquat.*  
626 *Toxicol.* 79, 366-375.

627 Lana, N.B., Berton, P., Covaci, A., Ciocco, N.F., Barrera-Oro, E., Atencio, A., Altamirano,  
628 J.C., 2014. Fingerprint of persistent organic pollutants in tissues of Antarctic notothenioid  
629 fish. *Sci. Total Environ.* 499, 89-98.

630 Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault,  
631 J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent  
632 organohalogen contaminants in arctic wildlife and fish. *Sci. Total Environ.* 408, 2995-3043.

633 Mintenbeck, K., Barrera-Oro, E.R., Brey, T., Jacob, U., Knust, R., Mark, F.C., Moreira, E.,  
634 Strobel, A., Arntz, W.E., 2012. 5 - Impact of Climate Change on Fishes in Complex Antarctic  
635 Ecosystems. in: Ute, J., Guy, W. (Eds.). *Adv. Ecol. Res.* Academic Press, 351-426.

636 Mirmigkou, S., de Boer, J., 2015. DDT and Metabolites. *The Handbook of Environmental*  
637 *Chemistry*, 1-24.

638 Murk, A., Legler, J., Denison, M., Giesy, J., Van de Guchte, C., Brouwer, A., 1996.  
639 Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah  
640 receptor active compounds in sediments and pore water. *Toxicol. Sci.* 33, 149-160.

641 Nash, S.B., 2011. Persistent organic pollutants in Antarctica: current and future research  
642 priorities. *J. Environ. Monitor.* 13, 497-504.

643 Nichols, J.W., Huggett, D.B., Arnot, J.A., Fitzsimmons, P.N., Cowan - Ellsberry, C.E., 2013.  
644 Toward improved models for predicting bioconcentration of well - metabolized compounds  
645 by rainbow trout using measured rates of in vitro intrinsic clearance. *Environmental*  
646 *Toxicology and Chemistry* 32, 1611-1622.

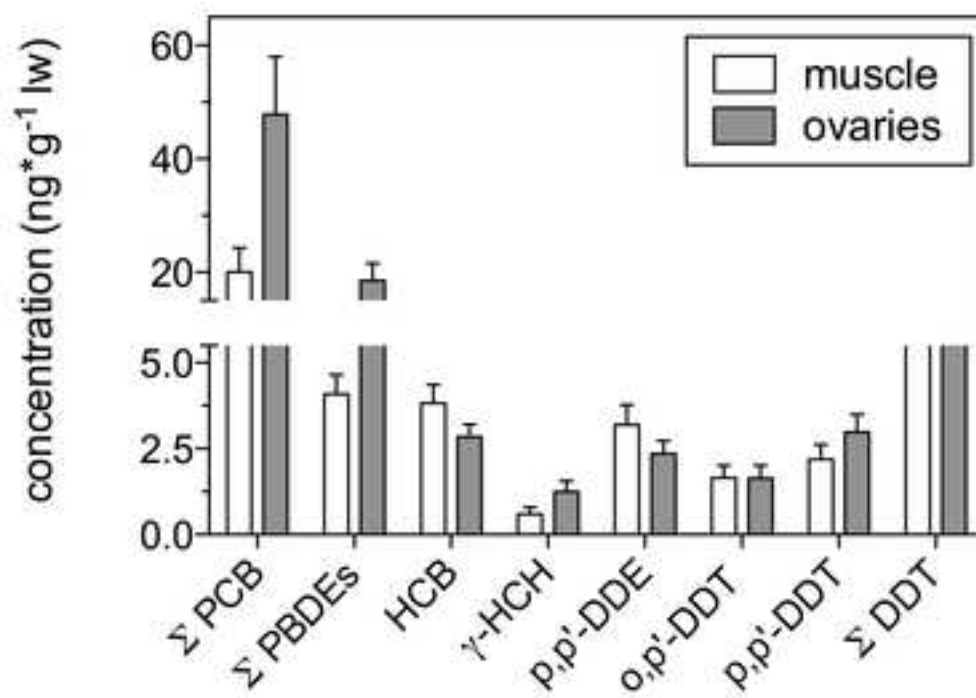
647 Renner, R., 2004. Small fish for healthy babies. *Environ. Sci. Technol.* 38, 262-263.

648 Schmid, P., Bogdal, C., Bl t hgen, N., Anselmetti, .S., wyssig, A., Hungerb hle r, K., 2010.  
649 The Missing Piece: Sediment Records in Remote Mountain Lakes Confirm Glaciers Being  
650 Secondary Sources of Persistent Organic Pollutants. *Environ. Sci. Technol.* 45, 203-208.  
651 Schmid, P., Kohler, M., Gujer, E., Zennegg, M., Lanfranchi, M., 2007. Persistent organic  
652 pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes  
653 in Switzerland. *Chemosphere* 67, 16-21.  
654 Sharma, C.M., Rosseland, B.O., Almvik, M., Eklo, O.M., 2009. Bioaccumulation of  
655 organochlorine pollutants in the fish community in Lake Årungen, Norway. *Environ. Pollut.*  
656 157, 2452-2458.  
657 Simonich, S.L., Hites, R.A., 1995. Global distribution of persistent organochlorine  
658 compounds. *Science* 269, 1851.  
659 Rigét, F., Bignert, A., Braune, B., Stow, J., Wilson, S., 2010. Temporal trends of legacy POPs  
660 in Arctic biota, an update. *Sci. Total Environ.* 408, 2874-2884.  
661 Storelli, M., Losada, S., Marcotrigiano, G., Roosens, L., Barone, G., Neels, H., Covaci, A.,  
662 2009. Polychlorinated biphenyl and organochlorine pesticide contamination signatures in  
663 deep-sea fish from the Mediterranean Sea. *Environ. Res.* 109, 851-856.  
664 Strobel, A., Burkhardt-Holm, P., Schmid, P., Segner, H., 2015. Benzo (a) pyrene metabolism  
665 and EROD and GST biotransformation activity in the liver of red-and white-blooded  
666 Antarctic fish. *Environ. Sci. Technol.* 49, 8022-8032.  
667 UNEP, 2002. Antarctica Regional Report. In: Chemicals, U.N.E.P. (Ed.). Regionally Based  
668 Assessment Of Persistent Toxic Substances.  
669 UNEP, 2008. Global Status Of DDT And Its Alternatives For Use In Vector Control To  
670 Prevent Disease. In: UNEP/POPS/DDTBP.1/2 (Ed.). Stockholm Convention on Persistent  
671 Organic Pollutants. UNEP, Geneva.  
672 UNEP/AMAP, 2011. Climate Change and POPs: Predicting The Impacts. Report of the  
673 UNEP/AMAP Expert Group. Geneva.  
674 Van den Berg, M., Birnbaum, L., Bosveld, A., Brunström, B., Cook, P., Feeley, M., Giesy,  
675 J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., 1998. Toxic equivalency factors (TEFs) for  
676 PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Persp.* 106, 775.  
677 van den Brink, N., Riddle, M., Heuvel-Greve, M.v.d., Allison, I., Snape, I., van Franeker,  
678 J.A., 2009. Correspondence on Geisz et al. Melting glaciers: a probable source of DDT to the  
679 Antarctic marine ecosystem. *Environ. Sci. Technol.* 43, 3976-3977.  
680 van den Brink, N.W., Riddle, M.J., van den Heuvel-Greve, M., van Franeker, J.A., 2011.  
681 Contrasting time trends of organic contaminants in Antarctic pelagic and benthic food webs.  
682 *Mar. Pollut. Bull.* 62, 128-132.  
683 Vives, I., Grimalt, J.O., Fernandez, P., Rosseland, B., 2004. Polycyclic aromatic  
684 hydrocarbons in fish from remote and high mountain lakes in Europe and Greenland. *Sci.*  
685 *Total Environ.* 324, 67-77.  
686 Wania, F., Mackay, D., 1993. Global fractionation and cold condensation of low volatility  
687 organochlorine compounds in polar regions. *Ambio*, 10-18.  
688 Weber, K., Goerke, H., 2003. Persistent organic pollutants (POPs) in Antarctic fish: levels,  
689 patterns, changes. *Chemosphere* 53, 667-678.  
690 Wolschke, H., Meng, X.-Z., Xie, Z., Ebinghaus, R., Cai, M., 2015. Novel flame retardants (N-  
691 FRs), polybrominated diphenyl ethers (PBDEs) and dioxin-like polychlorinated biphenyls  
692 (DL-PCBs) in fish, penguin, and skua from King George Island, Antarctica. *Mar. Pollut. Bull.*  
693 96, 513-518.  
694 Xie, ., M ller, A., Ahrens, L., Sturm, R., Ebinghaus, R., 2011. Brominated flame retardants  
695 in seawater and atmosphere of the Atlantic and the Southern Ocean. *Environ. Sci. Technol.*  
696 45, 1820-1826.  
697 Zennegg, M., Kohler, M., Gerecke, A.C., Schmid, P., 2003. Polybrominated diphenyl ethers  
698 in whitefish from Swiss lakes and farmed rainbow trout. *Chemosphere* 51, 545-553.

Figure 1

[Click here to download high resolution image](#)

*C. gunnari*



*C. aceratus*

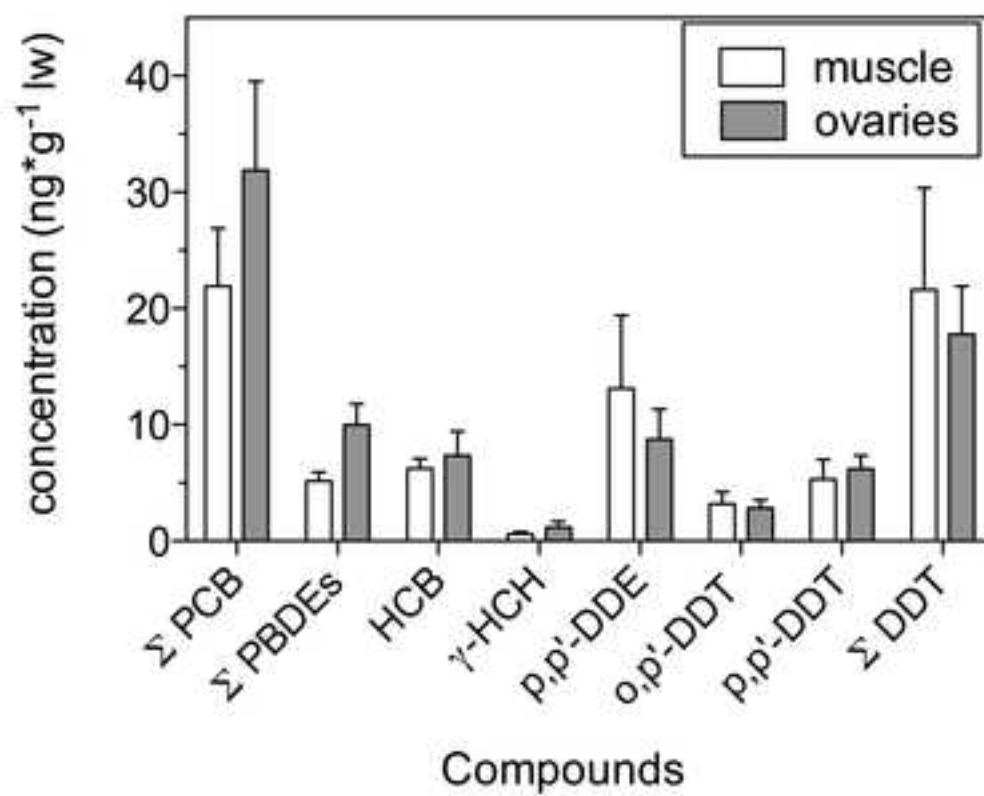
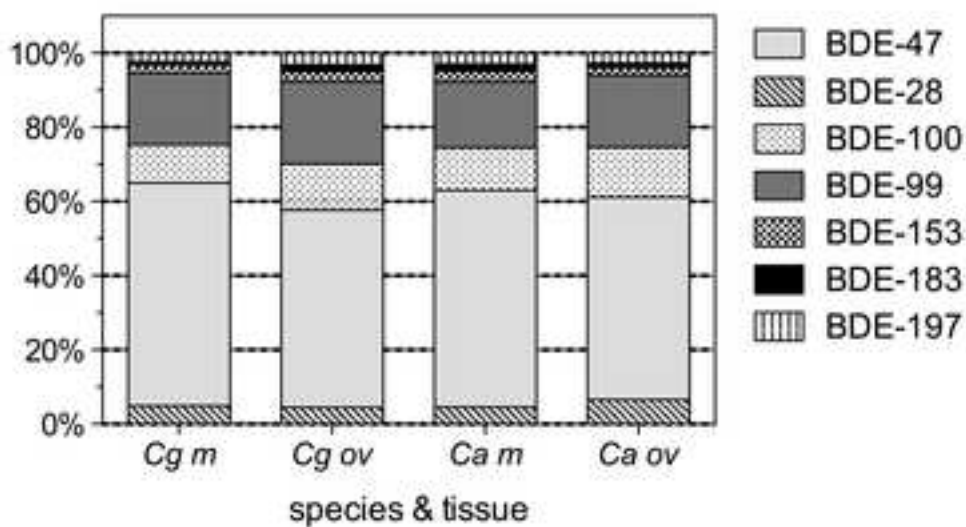
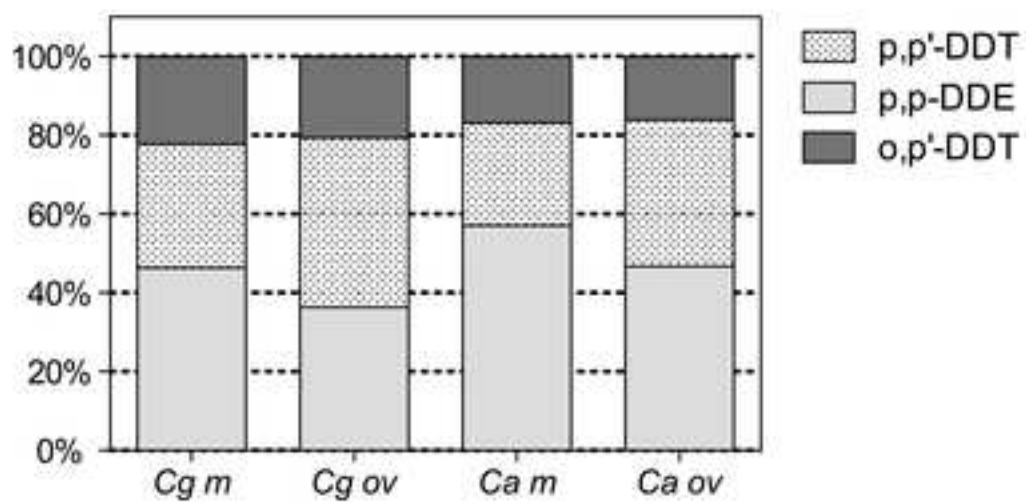
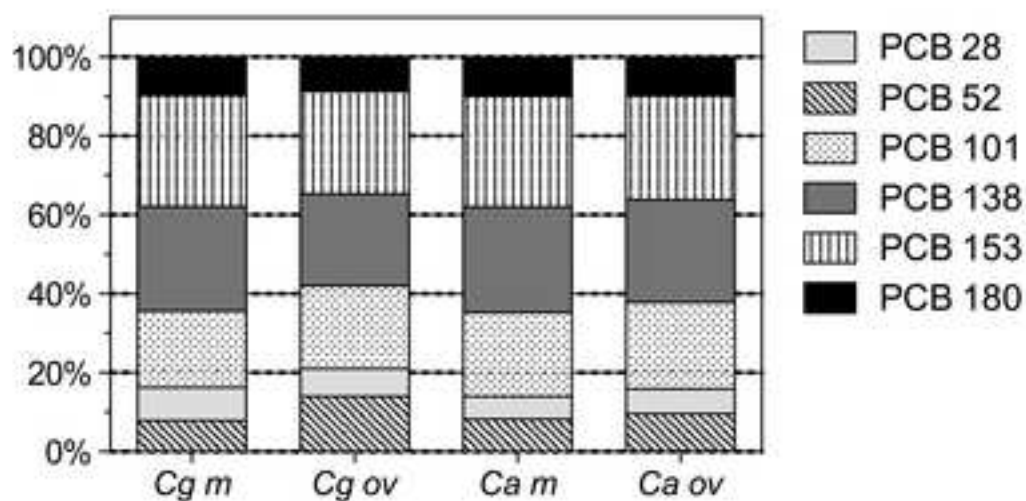




Figure 2

[Click here to download high resolution image](#)

### Congener composition



**Supplementary Material**

[Click here to download Supplementary Material: CHEM38908R1 Appendix A\\_Chemosphere.docx](#)